CHAPTER 1
INTRODUCTION

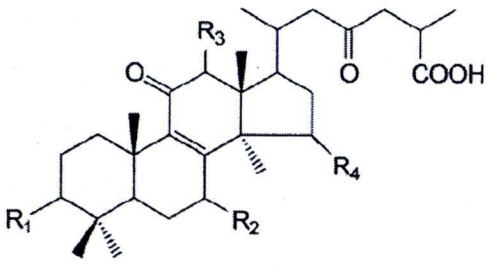
1.1 Statement and significance of the problem

The fruiting body of *Ganoderma lucidum* (Figure 1) known as Ling Zhi in China, one of the most famous traditional Chinese medicinal mushroom, has been used extensively for longevity and health promotion in China and other Asian countries for thousands of years [1-4]. Although it is still not clear about Ling Zhi's mechanism on longevity and health promotion, Ling Zhi has been used for the prevention or treatment of various conditions and diseases such as anorexia, neurasthenia, insomnia, migraine, allergy, asthma, bronchitis, gastritis, hepatitis, nephritis, arthritis, lupus erythematosus, diabetes, hypertension, hypercholesterolemia, cardiovascular problems as well as cancers [2-4].

Figure 1 *G. lucidum* (Ganodermataceae).

Modern investigations have revealed that Ling Zhi contains a variety of phyto-chemical compounds. One of the potent biologically active compounds that has been shown to possess diverse and potentially significant pharmacological activities is the bitter triterpenes [2, 3, 5]. Since the first discovery of ganoderic acids A and B, more

than 150 types of triterpenes have been isolated from various parts of Ling Zhi [3-6], among which ganoderic acids A and F (Figure 2) have received considerable attention due to their conspicuous pharmacological properties e.g., anti-hypertensive [7], anti-nociceptive [8], anti-oxidative [9], farnesyl protein transferase inhibitory [10], and hepatoprotective activities [11, 12], especially anti-cancer activity [13-16] which is the most attractive character of this medicinal mushroom. Ganoderic acid A has been reported to suppress growth and invasion of highly invasive human breast cancer cells via down-regulation of expression of cyclin-dependent kinase 4 (Cdk4) that regulates cell cycle G_1 phase progression, and via suppression of secretion of urokinase-type plasminogen activator (uPA) that implicates in tumor cell invasion and metastasis [15]. On the other hand, ganoderic acid F has exhibited anti-tumor and anti-metastatic activities through inhibition of angiogenesis [13] and alteration of proteins involving cell proliferation and/or cell death, carcinogenesis, oxidative stress, calcium signaling and endoplasmic reticulum stress [16].



Ganoderic acid A:

R_1=O, R_2= β-OH, R_3=H, R_4=α-OH

Ganoderic acid F:

R_1=R_2=R_4=O, R_3=β-OAc

Figure 2 Structures of ganoderic acids A and F.

Owing to these potential medical values, Ling Zhi has been increasingly cultivated and used as a health supplement and herbal medicine worldwide including Thailand [2, 4]. There are numerous commercial Ling Zhi preparations available in various brands and dosage forms derived from different parts of Ling Zhi such as capsules containing Ling Zhi extract or spore, tea bag, instant tea, and sliced fruiting bodies.

Although several lines of scientific data from *in vitro* and *in vivo* studies supporting Ling Zhi's various pharmacological activities have been extensively documented, quantitation of its biologically active compounds in various preparations

available in Thailand and the pharmacokinetic study regarding its bioactive compounds in human have not yet been reported. Therefore, the purposes of this study were to determine and compare the amount of ganoderic acids A and F, the potent biologically active compounds, in various Ling Zhi preparations available in Thailand, as well as to evaluate pharmacokinetics of ganoderic acids A and F and the influence of food on their pharmacokinetics after an oral administration of the water extract of MG2-strain Ling Zhi (MG2FB-WE), the product from Muang Ngai Special Agricultural Project under the patronage of Her Majesty Queen Sirikit, in healthy Thai male volunteers. This water extract of Ling Zhi in granular formulation is being under intensively investigated for its efficacy in the treatment of gynecologic and other cancers in the clinical trials conducted by the Faculty of Medicine, Chiang Mai University (CMU).

1.2 Literature review

1.2.1 History of *G. lucidum*

G. lucidum (Curt.: Fr.) P. Karst. is a species belonging to the family Polyporaceae (or Ganodermataceae) [3, 6]. The botanical name *Ganoderma* is established in 1881 by a Finnish botanist, Petter Adolf Karsten [2], and derived from the Greek (ganos means brightness, sheen and derma means skin), whereas *lucidum* is derived from the Latin (shiny or brilliant) [4]. Its fruiting body is well known as Ling Zhi (herb of spiritual potency or mushroom of immortality) in Chinese, Mannentake or Reishi (10,000 year-mushroom) in Japanese and Youngzhi in Korean [2-4].

In the nature, Ling Zhi grows at the base and on stumps of a variety of dying trees or deciduous trees especially oak, maple and mainly on plum trees in the eastern Asia. In the past, out of 10,000 such aged trees, perhaps two or three will have Ling Zhi growth, therefore it is very rare and very expensive. Since 1980, the production of Ling Zhi has been developed, it can now be cultivated, which makes it more accessible and affordable [4].

Ling Zhi has been widely used as a home remedy in traditional Chinese medicine (TCM) to preserve the human vitality and to promote longevity in China, Japan, and other Asian countries for over 4,000 years [1-4]. In the first pharmacopoeia of TCM, the Shen Nong's Materia Medica (*Shen Nong Ben Cao Jing*) published in the second century, Ling Zhi has been classified to be the superior medicine which is the herb of medicinal worth and without toxicity [2, 4, 17]. Li Shi-Zhen, a well known Chinese physician of the Ming Dynasty, also recorded its effectiveness and medical uses in the famously classic Compendium of Materia Medica (*Ben Cao Gang Mu*), and stated that taking Ling Zhi over the long period would result in a healthy body and longevity [4, 17, 18].

Evidence has accumulated regarding the medical utilization of Ling Zhi in the prevention and treatment of various kinds of ailments and chronic diseases such as anorexia, neurasthenia, insomnia, migraine, asthma, allergy, bronchitis, gastritis, hepatitis, nephritis, arthritis, hemorrhoid, constipation, dysmenorrhoea, lupus erythematosus, diabetes, hypertension, hypercholesterolemia, cardiovascular diseases and cancers [2-4].

1.2.2 Chemical constituents in *G. lucidum*

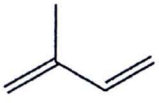
A diversity of chemical compounds has been identified from the fruiting body, mycelia, and spore of Ling Zhi such as triterpenes, sterols, polysaccharides, proteins, peptides, amino acids, nucleosides and nucleotides. Furthermore, alkaloids, vitamins, essential minerals and fatty acids have also been described [2-4]. A list of biologically active compounds and their pharmacological activities reported in various *in vitro* and *in vivo* studies is shown in Table 1. These diverse pharmacological activities may provide the potential medical benefits of Ling Zhi as previously described and triterpenes have received much interest as their well-known numerous and potentially significant pharmacological effects which predominate other constituents [2, 3, 5].

Table 1 Biologically active compounds reported in Ling Zhi and their pharmacological activities

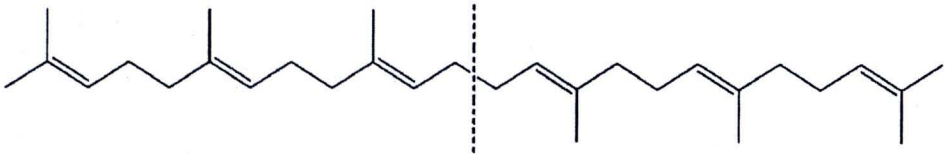
Active compound	Pharmacological activity	Reference
Adenosines	Anti-platelet aggregation	[19, 20]
Fatty acids	Anti-cancer	[21]
Polysaccharides	Anti-cancer and immunomodulatory	[17, 22-29]
	Anti-oxidative	[30, 31]
	Anti-viral	[32-34]
	Hepatoprotective	[35, 36]
	Hypoglycemic	[37-39]
	Neuroprotective	[40]
Proteins	Immunomodulatory	[41-44]
Triterpenes and related compounds	Anti-aging	[45]
	Anti-cancer	[13-16, 24, 26, 28, 29, 46-57]
	Anti-complement	[58, 59]
	Anti-histamine	[60]
	Anti-hypertensive	[7]
	Anti-inflammatory	[61, 62]
	Anti-nociceptive	[8]
	Anti-oxidative	[9]
	Anti-plasmodial	[63]
	Anti-platelet aggregation	[64]
	Anti-viral	[65-67]
	Bone protective	[68]
	Enzyme inhibitory	[10, 69-71]
	Hepatoprotective	[11, 12, 66, 72]
	Hypolipidemic	[73, 74]

1.2.3 Triterpenes

The term terpene is derived from the word "turpentine" (resin of pine trees). A single terpene unit is formed from two units of isoprene (C_5H_8), so that a triterpene consists of six isoprene units which may be linked together head to tail to form linear chains or arranged to form rings, and has the molecular formula $C_{30}H_{48}$ according to the isoprene rule or the C5 rule (Figure 3) [75].



Isoprene unit



Linear triterpene (e.g., squalene)

Figure 3 Structures of isoprene unit and linear triterpene.

Triterpenes are bitter compounds that cause Ling Zhi to have especially strong bitterness, not found in any mushroom [76]. Since the first isolation of two bitter triterpenes, ganoderic acids A and B, from the dried epidermis of Ling Zhi by Kubota *et al* in 1982, more than 150 types of triterpenes have been identified from its fruiting bodies, cultured mycelia, and spores by many research groups [3-6] and Paterson [3] suggests that more of these types of compounds will be detected in future studies.

Several studies showed that the differences in functional group and its position on the triterpene structure may be crucial for various biological activities of each triterpene [15, 59, 70] and considerable attention has been focused on ganoderic acids A and F (Figure 2).

1.2.4 Pharmacological activities of ganoderic acids A and F

Anti-cancer activity

The most attractive property of Ling Zhi is its anti-cancer activity. Kimura *et al* [13] demonstrated that ganoderic acid F, an active substance isolated from the triterpene fraction of *G. lucidum* fruiting body, can inhibit primary solid-tumor growth in the spleen, liver metastasis and secondary metastatic tumor growth in the liver in intrasplenic Lewis lung carcinoma-implanted mice. It also inhibits angiogenesis induced by matrigel (a soluble basement membrane extract of the Engelbreth-Holm-Swam tumor) stimulated with vascular endothelial growth factor and heparin in an *in vivo* model. This suggests that the anti-tumor and anti-metastatic activities of ganoderic acid F may be due to the inhibition of tumor-induced angiogenesis.

Recent *in vitro* study by Yue *et al* [16] exhibited the cytotoxicity of ganoderic acid F isolated and purified from fruiting bodies of *G. lucidum* on HeLa human cervical carcinoma cell line with half maximal inhibitory concentration (IC_{50}) value of 19.50 ± 0.60 μM possibly through altering proteins (e.g., eIF5A, 14-3-3 protein and peroxiredoxin) involved in cell proliferation and/or cell death, carcinogenesis, oxidative stress, calcium signaling and endoplasmic reticulum stress. Furthermore, study by Guan *et al* [14] showed that 17 known triterpenes including ganoderic acids A and F isolated from the fruiting bodies of *G. lucidum* have cytotoxicity against p388, HeLa, BEL-7402, and SGC-7901 human cancer cell lines with IC_{50} values in the range of 9.47-26.50 μM for ganoderic acid A and 9.62-19.10 μM for ganoderic acid F.

Ganoderic acid A has also been demonstrated to suppress growth (cell proliferation, colony formation) and invasive behavior (adhesion, migration, invasion) of highly invasive human breast cancer cells [15]. The anti-proliferative effect of ganoderic acid A may be mediated through the inhibition of transcription factor AP-1 resulting in the down-regulation of expression of Cdk4, whereas the anti-invasive effect may be mediated through the inhibition of NF-κB-dependent secretion of uPA. However, in the same study, ganoderic acid F has not shown any effect.

Anti-hypertensive activity

Angiotensin converting enzyme is responsible for catalyzing the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor causing hypertension. Morigiwa *et al* [7] found that ganoderic acid F and seven triterpenes isolated from 70% methanol extract of *G. lucidum* potentially inhibit this enzyme, especially ganoderic acid F, which has the highest inhibitory effect with an IC_{50} of 4.70 μM, whereas the IC_{50} values of other compounds are more than 10^{-5} M.

Anti-nociceptive activity

Koyama *et al* [8] demonstrated that among the active triterpenes from dichloromethane extract of *G. lucidum*, five ganoderic acids including ganoderic acid A have the anti-nociceptive effect against acetic acid-induced writhing response.

Anti-oxidative activity

Zhu *et al* [9] showed that the triterpene fraction containing ganoderic acids A as one of major ingredients has anti-oxidative effect against pyrogallol induced erythrocyte membrane oxidation and Fe (II)-ascorbic acid induced lipid peroxidation in a dose dependent manner and it is found to possess the highest effect when compares with polysaccharide fractions.

Enzyme-inhibitory activity

Farnesyl protein transferase (FPT) is the enzyme that catalyzes posttranslational farnesylation of cysteine residue near carboxyl terminus of the Ras oncoprotein, thus FTP inhibition has been suggested as a potential mechanism to interfere with the Ras-dependent cell transformation and represents a therapeutic strategy for the treatment of human cancers. Ganoderic acid A and its methyl ester (methyl ganoderate A) have been shown to have enzyme-inhibitory effect on FPT with IC_{50} values of 100 μM and 38 μM, respectively [10].

Hepatoprotective activity

β-glucuronidase is members of the glycosidase family of enzymes that catalyzes the breakdown of complex carbohydrates and it has been reported to be closely related to liver injury and liver cancer. Kim *et al* [11] demonstrated that ganoderic acid A isolated from the ether fraction of *G. lucidum* has potent β-glucuronidase-inhibitory activity and potent hepatoprotective effect against CCl_4-induced liver injury. This hepatoprotective effect is consistent with previous study but by different

mechanisms. Wang *et al* [12] demonstrated that *G. lucidum* extract containing ganoderic acid A as major component has the inhibitory effect on the proliferation of hepatic stellate cells activated by platelet-derived growth factor (PDGF) possibly through blocking the PDGF receptor phosphorylation, thereby indicating its efficacy for preventing and treating hepatic fibrosis.

1.2.5 Pharmacokinetic studies of ganoderic acids A and F

An enzyme immunoassay method has been developed for the determination of the ganoderic acid A level in rat plasma following the administration of purified ganoderic acid A dissolved in 5% DMSO-H_2O at doses of 5 and 50 mg/kg in rats after an overnight fasting. Blood samples were collected from a tail vein at 10, 20, 40 min, 1, 2, 4 and 8 h. The results showed that ganoderic acid A appears as early as 5 min after administration. The maximal concentrations (C_{max}) of ganoderic acid A are 0.037 ± 0.008 and 0.595 ± 0.125 μg/mL at doses of 5 and 50 mg/kg, respectively. The time to reach maximum concentration (T_{max}) is about 18 min for both doses and then the level decreases sharply. The area under the plasma concentration-time curves from 0-480 min (AUC_{0-480}) are 3.29 ± 0.98 and 27.76 ± 0.13 μg.min/mL for 5 and 50 mg/kg doses, respectively, which proportionally increase with the administered dose. In comparison with the AUC_{0-480} values at a dose of 5 mg/kg between oral and intravenous administration of ganoderic acid A, the absolute bioavailability is determined to be 0.10, indicating that approximately 10% of oral dose of ganoderic acid A is absorbed into systemic circulation [77].

Xue *et al* [78] investigated the absorption of total triterpenes from *G. lucidum* into rat plasma by using high performance liquid chromatography with diode array detection (HPLC-DAD) and liquid chromatography-mass spectrometry (LC-MS) methods. Male Sprague-Dawley rats were fasted for 12 h with free access to water, and then they were orally administered the dosage of 800 mg/kg of total triterpenes dissolved in castor oil. The blood samples were collected into heparinized tubes 4 h thereafter. The results showed that five triterpenes such as ganoderic acid A, B, C_2, C_6, and G can be simultaneously detected in rat plasma after oral administration of total triterpenes from *G. lucidum*. However, the authors suggested that some of the ganoderic acids may be transformed into other related structures as metabolites.

A recent study also evaluated the absorption of ganoderic acids A and F after oral administration of *G. lucidum* extract containing 13.4% triterpenes into rat plasma by using HPLC-MS analysis. Female Sprague-Dawley rats received one dose of *G. lucidum* extract by gastric gavage (500 mg/kg) and aliquots of plasma were collected at 0, 15, 30, 45, 60, 90 and 120 min by retrorbital venipuncture method into heparin-containing tubes. T_{max} of ganoderic acids A and F is 90 min and then rapidly decreases within the next 30 min, therefore it has been suggested that ganoderic acids A and F can be detected in rat plasma within a short period of time after an oral application [79].

1.2.6 Clinical studies of *G. lucidum*

Most clinical studies have evaluated the efficacy of *G. lucidum* extract which contains many biologically active compounds or its polysaccharide fraction. However, the clinical study to investigate the effects of triterpene fraction is still lacking.

Study in healthy subjects

Wachtel-Galor *et al* [80] assessed the effects of *G. lucidum* extract (1.44 g/day) on a range of biomarkers of anti-oxidant status, coronary heart disease risk, DNA damage, immune status and inflammation, as well as markers of hepatic and renal toxicity and genotoxicity in healthy subjects. Although the results showed decreasing in plasma lipids (triacylglycerol, total and LDL-cholesterol) and increasing in anti-oxidant capacity in urine after a 4-wk period of Ling Zhi supplementation, these changes were not significant. Moreover, other biomarkers such as high-sensitivity C-reactive protein, plasma HDL-cholesterol, CD4 : CD8 ratio, leukocytes, as well as anti-oxidant status (plasma ascorbic acid and α-tocopherol, erythrocyte superoxide dismutase and glutathione peroxidase), have also shown no significant improvement. However, evidence of hepatic and renal toxicity including genotoxicity has not been observed.

Wicks *et al* [81] also evaluated the safety, tolerance and effect on immune function after oral administration of *G. lucidum* extract (4 g/day twice daily for 10 days) in healthy subjects. The results showed no significant changes in CD4, CD8, CD19 and CD56 levels after administration and no adverse effects have been observed. In addition, a randomized double-blind placebo-controlled study by

Kwok *et al* [82] demonstrated that oral administration of *G. lucidum* extract (1.50 g/day three times daily for 4 wk) is not associated with the impairment of platelet and global hemostatic functions according to the following tests; routine hematology tests, von Willebrand ristocetin cofactor activity, fibrinogen concentration, thrombelasto-graphy and platelet function analyzer. These findings indicated that the oral administration of Ling Zhi is unlikely to increase risk of bleeding in healthy volunteers as previously reported in *in vitro* and *in vivo* studies.

Study in patients with neurasthenia

Tang *et al* [83] revealed that patients with neurasthenia treated with Ganopoly, a polysaccharide fractions extracted from *G. lucidum*, at 5.40 g/day three times a day for 8 wk have a marked lowering in the Clinical Global Impression severity score and a significant improvement in visual analogues scales for the sense of fatigue and well-being.

Study in patients with herpes simplex and herpes zoster virus infections

In a study to evaluate the efficacy in patients with herpes genitalis and labialis who suffering from recurrent herpes outbreaks for more than 1 y and strongly tolerant to the standard treatment, reported that herbal mixtures comprising *G. lucidum* have a significant improvement in the recovery time with the mean duration to relieve symptoms from herpes genitalis of 10.9 and 4.9 days without and with treatment, respectively. Similarly, the time required for symptomatic relief from herpes labialis is 7.8 and 4.0 days without and with treatment, respectively. These results suggested that herbal mixture containing *G. lucidum* can provide fast and effective relief from the symptoms of recurrent herpes genitalis and labialis [84].

Moreover, Hijikata *et al* [85] found that oral administration of hot water soluble extracts of *G. lucidum* at 36-72 g dry weight/day can decrease pain in two patients with post-herpetic neuralgia (PHN) that resistant to the standard therapy and two other patients with severe pain due to herpes zoster infection. Their further study also showed that oral administration of hot water extracts of an herbal formula containing *G. lucidum* 0.75 g weight/dose can reduce pain in five patients suffering from herpes zoster. Pain relief starts within a few days and has almost completely disappeared within 10 days. Moreover, no patient develops PHN over one year of follow-up [86].

Study in patient with hepatitis B virus infection

Ganopoly (1.80 g/day three times daily for 12 wk, then 13 wk followed up) has been also done in a double-blind, randomized and multicenter study in patients with chronic hepatitis B according to the positive value of hepatitis B virus-DNA. The results exhibited that Ganopoly is well tolerated and effective with 33% (17/52) of treated patients have normal aminotransferase values and 13% (7/52) have hepatitis B surface antigen (HBsAg) cleared from serum within the 6 month study period, whereas none of the controls have normal enzyme values or have lost HBsAg [87].

Study in patients with type 2 diabetes mellitus

Seventy-one patients with confirmed type 2 diabetes treated with Ganopoly (5.40 g/day three times daily for 12 wk) have a significant decrease in the mean post-prandial glucose to 11.80 mmol/L when compared to the placebo group [88].

Study in patients with urogenitary diseases

A study to evaluate the safety and efficacy of the ethanol extract of *G. lucidum* in men with lower urinary tract symptoms showed that oral administration at a dose of 6 mg once a day for 12 wk has a significantly better improvement than placebo in total International Prostate Symptom Score with no major adverse effects. However, no changes in quality of life scores, peak urinary flow, mean urinary flow, residual urine, prostate volume, prostate-specific antigen or testosterone levels [89].

Furthermore, Futrakul *et al* [90] demonstrated that nephrotic patients with focal segmental glomerulosclerosis and persistent proteinuria treated with crude extract of *G. lucidum* 0.75-1.10 g/day plus vasodilators, consisting of enalapril (0.50-1.00 mg/kg/day), isradipine (5-10 mg/day) and dipyridamole (75-225 mg/day), for 1 y have a significant decrease in proteinuria and a slight but not statistically significant increase in creatinine clearance, whereas group of patients treated with vasodilators alone have not shown any statistically significant improvement.

Study in patients with cancers

Gao *et al* [91] exhibited that oral Ganopoly treatment at 5.40 g/day three times daily before meals for 12 wk enhance the immune responses in 34 patients with advanced-stage cancer by significant increase in the mean plasma concentrations of interleukin (IL)-2, IL-6 and interferon (IFN)-γ, number of CD56 cells including NK activity, whereas there is significant decrease in the levels of IL-1 and tumor necrosis

factor (TNF)-α when compared to baseline values. However, other biomarkers for immune function e.g., the numbers of CD3, CD4, CD8 as well as CD4 : CD8 T cell ratios have remained unchanged. Ganopoly administration (5.40 g/day three times daily for 12 wk) has also been reported to have palliative effect on cancer-related symptoms such as sweating and insomnia in many patients with advanced solid cancer, whereas the objective responses (complete or partial disappearance of all tumor masses) have not been found in this study. Besides, five adverse events of mild severity have been recorded, 3 of which are gastrointestinal problems (2 cases of nausea, and 1 case of diarrhea) [92].

A randomized double-blind, placebo-controlled, multicenter trial to assess the efficacy of Ganopoly (1.80 g/day three times daily for 12 wk) in 68 patients with histologically confirmed advanced lung cancer demonstrated that 32 assessable patients treated with Ganopoly result in a significant increase in the quality of life (Karnofsky score) in 16 patients, 4 patients obtain significant increase in the control group with 29 assessable patients. However, 3 episodes of mild toxicity (2 cases of nausea and 1 case of insomnia) have appeared in the treatment group [93]. Subsequent study also investigated the effects of Ganopoly (5.40 g/day for 12 wk) on immune functions in 36 patients with advanced lung cancer. The results demonstrated that treatment with Ganopoly has not shown a significant change in the mean mitogenic reactivity to phytohemagglutinin (PHA), mean counts of CD3, CD4, CD8, and CD56, mean plasma concentrations of IL-2, IL-6, and IFN-γ, or NK activity in the patients. However, some cancer patients have demonstrated obviously modulated immune functions. These findings suggested that cancer patients may be responsive to Ganopoly in combination with chemotherapy or radiotherapy [94].

Chen *et al* [95] found that 41 patients with advanced colorectal cancer treated with Ganopoly (5.40 g/day three times daily, orally before meals for 12 wk) have no statistical significant changes in mitogenic reactivity to PHA, counts of CD3, CD4, CD8 and CD56 lymphocytes, plasma concentrations of IL-1, IL-2, IL-6, IFN-γ and TNF-α including NK activity. In addition, a recent study by Oka *et al* [96] assessed the effect of water-soluble extract from culture medium of *G. lucidum* mycelia (1.50 g/day for 12 months) in 123 patients with colorectal adenomas. The authors found that patients received treatment have a significant decrease in the number (-0.42) and

the total size of adenomas (-1.40 mm). Conversely, these variables in control group have been reported a significant increase in the number of adenomas (0.66) and total size (1.73 mm). The results suggested that *G. lucidum* water extract is effective to suppress the development of colorectal adenomas, precancerous lesions of the large bowel.

1.2.7 Toxicity and safety of *G. lucidum*

In animal experiments, *G. lucidum* extracts show a very low toxicity, however, there are few reports on the long-term adverse effects. The aqueous extract of *G. lucidum* administered to mice (5 g/kg orally for 30 days) has shown no changes in body weight, organ weight, or hematological parameters. Similarly, polysaccharide fraction at the same dose has no lethal or serious adverse effects [97]. In addition, an oral dose of 10 g/kg of *G. lucidum* produces no changes in the estrus cycles of ovariectomized female mice and no increase in the weight of levator cavernosa and testicles in male mice. The lethal dose $(LD)_{50}$ in mice of the reflux percolate is 38.30 ± 1.05 g/kg. No organ toxicity has been found in rabbits taking a syrup preparation of *G. lucidum* (progressive oral doses of 4-140 mL/kg daily for 10 days), or in dogs (2 and 4 mL/kg per oral daily for 10 days) [4].

Moreover, daily intragastric administration of an alcoholic extract at doses of 1.20 and 12 g/kg for 30 days has been reported no signs of toxicity in young rats in major organs, hepatic function, growth, or development. Although toxicity are absent in dogs administered an alcoholic extract intragastrically daily at dose of 12 g/kg for 15 days and at 24 g/kg for 13 days, dogs display lethargy [4]. A study to assess the acute toxicity of *G. lucidum* prepared as a freeze-dried powder extract demonstrated that male mice taking the extract solution at a dosage equivalent to the one commonly recommended by manufacturers of commercial concentrated extracts, have shown no evidence of acute toxicity and no significant differences in the serum concentrations of urea, glutamic-oxaloacetic transaminase, as well as glutamic-pyruvic transaminase when compared to controls. No abnormalities have been found in histological examinations of liver and kidney, organ weight (liver, kidney, heart, lung, and spleen), or organ/body weight ratios when compared to the control [98].

In several clinical studies showed that the administration of Ling Zhi extract is well tolerated and there are no serious adverse events or toxicities in healthy subjects [80, 81] as well as patients [87, 89, 92, 93, 99], although mild grade adverse events such as rash, neuralgia, insomnia, fatigue, nausea, diarrhea, constipation, or high blood pressure [89, 92, 93, 99] have been reported. Noguchi *et al* [89] have reported one case of serious adverse event (chest pain) in patient treated with 6 mg once daily of ethanol extract from *G. lucidum*.

There are reports of two cases of hepatotoxicity related to a formulation of *G. lucidum*. The first case of hepatotoxicity was reported from Hong Kong in 2003. The patient was a 78-year-old Chinese lady having hypertension treated with felodipine for two years and baseline liver biochemistry in routine follow-ups for hypertension was normal. She had no history of significant alcohol consumption. She used to take traditionally boiled Ling Zhi slices regularly for at least one year as a health supplement and changed to powder form for one month before having the hepatotoxic episode. Physical examination only revealed marked jaundice with abnormal liver biochemical tests, whereas the complete blood counts, eosinophil count including prothrombin time were normal. Ultrasonography and computerized tomography of the liver were then performed. Both investigations did not present any feature of biliary obstruction or gallstone. Immunoglobulin titers were within normal range as well as hepatitis virus markers were all negative. However, histology of the liver biopsy showed the presence of eosinophil infiltrations in the portal tracts and histological features were compatible with drug-induced liver damage which suggests that the mechanism is mediated through an immune-allergic reaction. After hospital admission, she was asked to stop taking this powder formulation of Ling Zhi and all the liver biochemical parameters gradually improved to nearly normal levels 5 months later. The authors suggest that other ingredients of the powder formulation are most likely to be the cause of hepatotoxicity since the addition of other plants or ingredients in the preparation of Chinese herbal medicines is very common [100].

In 2005, the second case of fatal toxic hepatitis associated with Ling Zhi powder was reported. The patient was a 47-year-old female with a history of schizophrenia on treatment with lithium, perphenazine, and trihexyphenidil long-term. Her previous liver biochemical profiles were within normal limits. She used to take traditionally

boiled Ling Zhi slices for several years without any adverse effect. She changed to take one capsule of Ling Zhi powder containing 400 mg of Ling Zhi extract per day for two months before having the episode of jaundice and coma. On admission, the serological tests for hepatitis virus markers were all negative, as well as the blood levels of lithium, acetaminophen, and perphenazine were below the toxic levels. However, the liver biochemistry and prothrombin time were markedly higher than normal limits. Histology of the liver biopsy was performed and interpreted as toxic or drug-induced hepatitis causing acute hepatic failure. The authors propose that the induction of cytochrome P450 enzymes by other therapeutic agents concurrently used may synergistically enhance the production of toxic metabolite derived from Ling Zhi powder. In addition, the hepatotoxicity from Ling Zhi powder have to be closely monitored since the preparation in powder formulation is increasing in popularity due to its convenience in consumption [101].

1.3 Purposes of the study

The purposes of the present study were to

1. Determine and compare the contents of ganoderic acids A and F in various Ling Zhi preparations available in Thailand.
2. Evaluate the pharmacokinetics of ganoderic acids A and F after a single oral administration of MG2FB-WE in healthy Thai male volunteers.
3. Assess the influence of food on the pharmacokinetics of ganoderic acids A and F after a single oral administration of MG2FB-WE in healthy Thai male volunteers.