CHAPTER II
LITERATURE REVIEWS

2.1 Traditional phytomedicine of *Andrographis paniculata*

Andrographis paniculata Nees. (Family Acanthaceae) (Figure 1), named in Thai "Fha-ta-lai-jorn", has traditionally employed for centuries in Asia and Europe as a folklore remedy for a wide spectrum of ailments (Jarukamjorn, 2008). It is also known as "King of Bitters" of Acanthaceae family. This plant has been widely used against different diseases including cold, hypertension, diabetes, etc. (Barilla, 1999). *A. paniculata* was also recommended in Charaka Samhita (Ayurveda) dating to 175 B.C. for treatment of jaundice along with other plants in multi-plant preparations (Sharma, 1983). According to a database of compounds found in plants used in traditional Chinese medicine (NiceData Software, 2004), *A. paniculata* is used to treat snake bite and eczema, two conditions relating to the skin. The herb is used to rid the body of heat, as in fever, and to dispel toxins from body (Deng, 1978).

Figure 1 *A. paniculata* Nees

In Malaysia, decoction from the aerial parts of *A. paniculata* is used to treat cold, hypertension, diabetes, cancer, malaria, and snake bite (Perry, 1980). In Europe, this plant has been used to prevent and treat common cold (Caceres et al., 1997).

All parts of this herb are medicinally important in the traditional system of medicine in India and have been extensively used as a febrifuge, bitter tonic, stomachic, antihelminthic, and hepatoprotective (Mandal et al., 2001). In Thailand, this plant was selected by Ministry of Public Health as one of the medicinal plants in "The National List of Essential Drugs A.D. 1999" (List of Herbal Medicinal Products) (Jarukamjorn, 2008).

A. paniculata is nowadays incorporated into a number of herbal medicinal preparations such as dry leaves, powder, capsule, and Thai traditional pill (Figure 2).

Figure 2 Medicinal preparations of *A. paniculata*

2.1.2 Chemical constituents of *A. paniculata*

Major constituents of *A. paniculata* include diterpenoids, flavonoids, and polyphenols. Among the compounds extracted from the plant *A. paniculata*,

andrographolide (Figure 3), (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-napthalenyl]ethylidene] dihydro-4-hydroxy-2(3H)-furanone), is the major one in terms of bioactive property and abundance (Chao & Lin, 2010).

The content of andrographolide in *A. paniculata* plants is 2.09 – 5.59 percentage of the dry-weight, due to various seasons of harvesting and regions of plant cultures (Patarapanich et al., 2007; Jain et al., 2000). Beside andrographolide, other constituents such as 14-deoxyandrographolide, isoandrographolide, andrograpanin, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, andrographiside, stigmasterol, 5-hydroxy-7,8-dimethoxyflavone, several diterpenoids, and serveral flavonoids have been found in the plant *A. paniculata* (Chem & Liang, 1982; Shama et al., 1992; Siripong et al., 1992; Cheung et al., 2001).

2.1.3 Pharmacological activities of *A. paniculata* and its constituents

2.1.2.1 Antioxidative property

The methanol and water crude extract of *A. paniculata* leaves inhibited lipid peroxidaion in the rat homogenated-livers (Akowuah et al., 2006). The lipid peroxidation inhibitory activity varied from 55.67 to 63.82% and 33.78 to 33.77% for the methanol and the water extracts, respectively. The methanol extracts exhibited free radical scavenging activity ranging from 45.67 to 53.82%, but the water extracts exhibited poor free radical scavenging activity ranging from 25.29 to 28.77%. The variation of anti-lipid peroxidation and free radical scavenging activity depends on the concentration of andrographolide and 14-deoxy-11,12-didehydroandrographolide in those extracts (Akowuah et al., 2006).

2.1.2.2 Antimicrobial activity

Andrographolide and neoandrographolide were reported to use in Bacillary dysentery disease (Fabricant and Farnsworth, 2001). Water decoction made from the leaves of *A. paniculata* has been reported to completely *Dipetalonema reconditum* microfilaria *in vitro* (Datta & Sukul, 1982). Extract of *A. paniculata* showed microfilaricidal activity at different concentrations, ranged from 1 µg/mL to 10 mg/mL within 24 h, by evaluation of relative microfilarial motility (Merawin et al., 2010). Methanolic extract of *A. paniculata* at concentration of 0.8 to 2.5 mg/mL completely inhibited development of *Plasmodium falciparum* at 48 h-incubation time,

while chloroform extract inhibited parasite growth at a concentration of 0.1 mg/mL at 24 h (Rahman et al., 1999). Xu and colleagues (2006) reported antimicrobial activity of ethanolic extract of *A. paniculata* on two human pathogens including *Legionella pneumophila* and *Bordetella pertussis*. Aqueous extract of *A. paniculata* was tested *in vitro* against adult worms of subperiodic *Brugia malayi* (Zaridah et al., 2001) in which relative movability of *B. malayi* was lowered, indicating antifilarail activity of *A. paniculata* extract.

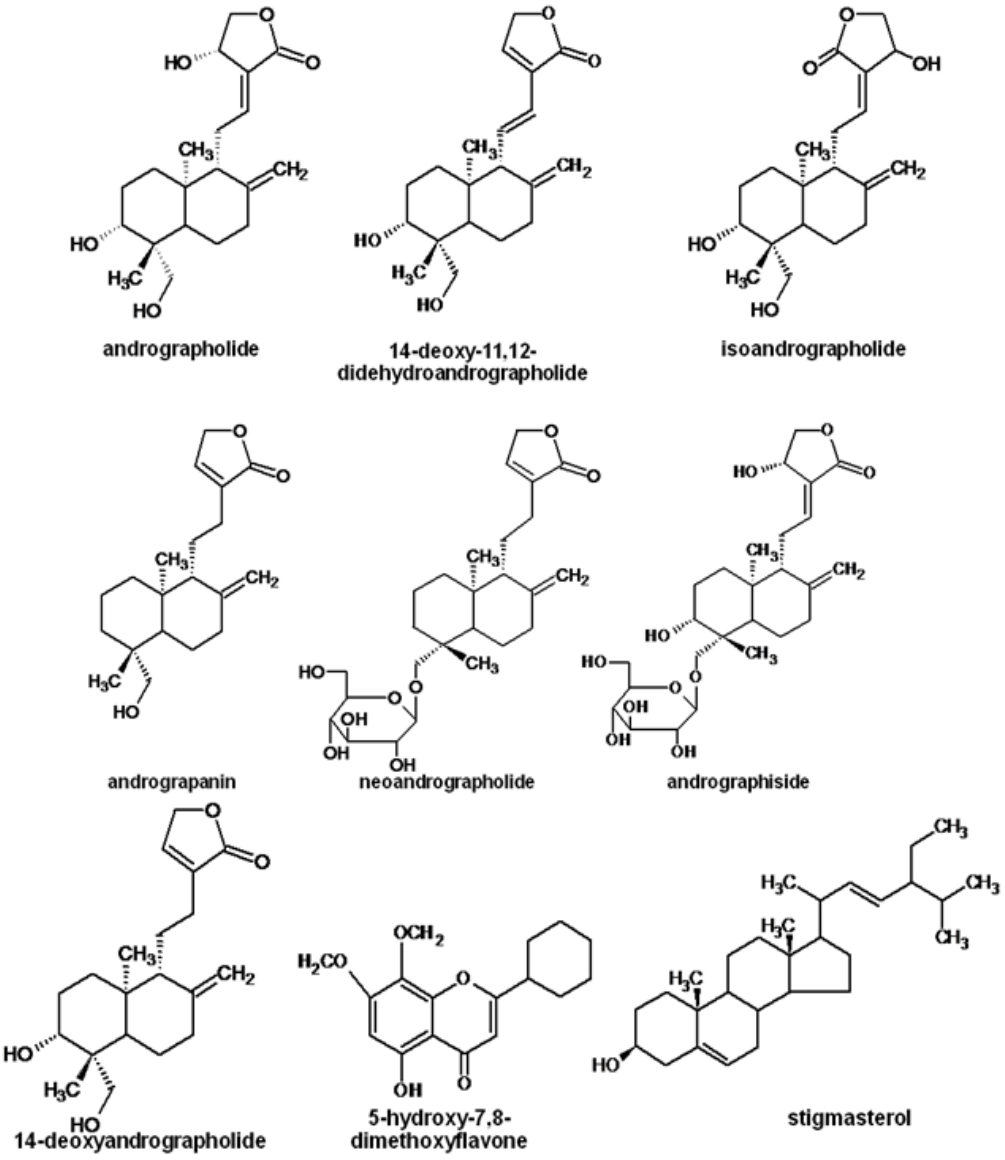

Figure 3 Active constituents in *A. paniculata*

2.1.2.3 Cardiovascular effects

The extract of *A. paniculata* extract has been employed for treatment of hypertension in Malaysian traditional medicine. Zhang and Tan (1996, 1997) reported that *A. paniculata* extract transiently reduced the blood pressure of rats. Furthermore, they found that pure andrographolide did not produce a similar reduction in blood pressure, suggesting that other compounds might elicit this effect. Recently, 14-deoxyandrographolide and 14-dideoxy-11,12-didehydroandrographolide were reported to possess hypotensive activities (Zhang & Tan, 1998; Zhang et al., 1998; Zhang & Tan, 1999). Moreover, bisandrographolide reduced blood pressure via the activation of transient receptor potential channel, TRPV4 (Smith et al., 2006). Zhao and Fang (1991) found flaovone extract from the root of *A. paniculata* inhibited platelet aggregation and production of thromboxane B2, which might be a benefit of *A. paniculata* for prevention of myocardial infarction.

2.1.2.4 Anti-cancer activity

The methanolic extract of *A. paniculata* leaves showed significant cytotoxic activity against the growth of human epidermoid carcinoma of nasopharynx (KB) and P388 lymphocytic leukemia tumor cell cultures (Siripong et al., 1992). Intraperitoneal administration of *A. paniculata* extract and andrographolide significantly inhibited the B16F-10 melanoma cell induced capillary formation in C57BL/6 mice. *A. paniculata* extract and andrographolide inhibited tumor specific angiogenesis by down regulating various proangiogenic molecules such as vascular endothelial growth factor, nitric oxide, and proinflammatory cytokines, as well as up-regulating antiangiogenic molecules like interleukine-2 and tissue inhibitor of metalloproteinases-1 (Sheeja et al., 2007).

Moreover, andrographolide showed inhibitory action on cell-cycle progression in human colorectal Lovo cell growth at G1–S phase arrest, and exerted inducible expression of p53, p21, and p16 that, in turn, repressed the activity of Cyclin A/Cdk2 and/or Cyclin D1/Cdk4, as well as Rb phosphorylation. These observations revealed cancer chemotherapeutic potential of andrographolide (Shi et al., 2008; Shi et al., 2009).

2.1.2.5 Hepatoprotective activity

Singha and colleagues (2007) reported hepatoprotective activity of *A. paniculata* extract and andrographolide in mice. Andrographolide and arabinogalactan proteins, which isolated from *A. paniculata*, at different dose ranged from 62.5 to 500 mg/kg showed the potential to prevent ethanol-induced toxicity in mouse liver and kidney (Singha et al., 2007). Andrographolide was more biologically potent than silymarin (a standard hepatoprotective agent) against paracetamol-induced liver damage (Visen et al., 1993). Moreover, andrographolide has been reported to show hepatoprotective activity in mice against carbon tetrachloride and paracetamol intoxication (Handa & Sharma, 1990).

2.1.2.6 Analgesic and antipyretic activities

Madav and colleagues (1995) reported analgesic activity of andrographolide (300 mg/kg, p.o.) on acetic-induced writhing response in mice and the Randall-Selitto test in rats. The extract of *A. paniculata* (100-300 mg/kg, p.o.) significantly potentiated pentobarbitone-induced sleeping time and lowered normal body temperature in mice (Mandal et al., 2001) and significantly reduced acetic acid-induced writhing response in mice, while no analgesic response was observed in the tail clip test (Mandal et al., 2001).

2.1.2.7 Anti-inflammation activity

The dramatic reduction of peritoneal deposition of neutrophils induced by cytokines and LPS was observed in andrographolide treated-cell. Moreover, NF-kB activity was potently inhibited by andrographolide (Xia et al., 2004), leading to reducing of pro-inflammatory proteins such as cyclooxygenase-2. In addition to the long history of using *A. paniculata* and andrographolide as remedy for inflammatory disorders, previous observations that andrographolide suppressed nitric oxide (NO) production and down-regulated leukocyte integrin Mac-1 (αMβ2, CD11bCD18), leading to inhibition of neutrophil adhesion and transmigration, also strongly supported the anti-inflammation activity of andrographolide (Shen et al., 2000; Shen et al., 2002; Batkhuu et al., 2002).

From the numerous beneficent pharmacological activities of *A. paniculata* and its components mentioned above revealed that *A. paniculata* and andrographolide have high potential to develop as a modern medicine, following use in clinical

practice in the future. However, extensive studies on pharmacological aspects are not enough to affirm safety in human. Toxicology and pharmacokinetics including metabolism of *A. paniculata* and andrographolide are needed to be absolutely unraveled to assure the safety of using *A. paniculata* and andrographolide as alternative medications or health supplements in human.

2.1.4 Toxicology of *A. paniculata* and andrographolide

Only two reproductive toxicological studies of *A. paniculata* and andrographolide were reported by Akbarsha and colleagues (1990, 2000). The extract of *A. paniculata* leaves showed arrest of spermatogenesis in male albino rats (Akbarsha & Manivannan, 1993). Oral administration of andrographolide (25-50 mg/kg/day) in rats for 48 days caused a decrease of sperm count, abnormalities of sperm, and lost motility of spermatozoa. Andrographolide affected spermatogenesis by preventing cytokinesis of the dividing spermatogenic cell lines (Akbarsha & Murugaian, 2000). On the other hand, other studies showed the safety of *A. paniculata* and andrographolide. A study of Burgos and colleagues (1997) reported that no testicular toxicity was found in male Sprague Dawley rats with the treatment of 20, 200, and 1000 mg/kg of *A. paniculata* extract over 60 days-period evaluated by weight of reproductive organ, testicular histology, ultra-structural analysis of Leydig cells, and testosterone levels. Recently, the administration of 60 mg of andrographolide to healthy male volunteers for 10 days did not decrease male fertility determined by volume of ejaculate, number of sperms, and active spermatozoids (Mkrtchyan et al., 2005), while acute oral toxicity of *A. paniculata* was reported in female rats orally treated with 5 g/kg of *A. paniculata* for 14 days (Chandrasekaran et al., 2009). *A. paniculata* did not show toxic effects on body weight and any pathological changes in rats. Moreover, genotoxicity of *A. paniculata* was determined using Ames test, chromosome aberration, and micronucleus assay. At the concentrations of 8.8 – 5,000 µg/mL of *A. paniculata* were confirmed not to induce mutation and clastogenicity (Chandrasekaran et al., 2009).

Though several toxicological studies of *A. paniculata* and andrographolide indicated that using this herb was weakly toxic, there were insufficient to confirm safety for long term use. The numbers of toxicological study are still limited,

compared to pharmacological activities. In general, an herbal medicine is employed in combination with the prescribed modern medication aiming to improve status of the disease, or to positively obtain a synergistic or additive effect, and with a belief that an alternative medicine is normally non-toxic, leading to long term consuming. During use of *A. paniculata* or andrographolide as alternative medications or health supplements, those people have possibly been prescribed other modern medications or exposed to other chemicals, particularly carcinogens, in which metabolism pathways of those compounds might interfere each other. Therefore, the studies of impacts of *A. paniculata* and andrographolide on CYPs expression are necessary for indicating safety/adverse/toxic information. Moreover, understanding the way in which herb, drugs, or xenobiotics induce or inhibit major metabolizing enzymes, especially cytochrome P450s, is biologically relevant and ultimately leads to better models for screening and predicting herb-drug interactions.

2.2 Cytochrome P450 and CYP1A

2.2.1 Cytochrome P450 superfamily

Humans daily expose exogenous compounds present in the environment. Metabolism is a process to convert chemical compounds to be more water-soluble, leading to be easily excreted in urine or feces. Metabolism is divided into two phases, phase I and II. Phase I metabolism performs multiple types of reactions such as oxidation, reduction, and hydrolysis. Phase II metabolism performs drug-conjugation such as glucuronide, sulfate, and glutathione conjugations, to become more soluble and then lead to excretion. Cytochrome P450s (CYPs) is a large and diverse group of hemethiolate isoenzyme that catalyses monooxygenase reaction in the phase I metabolism. CYPs require a cytochrome P450 reductase to transfer electrons from β-nicotinamide adenine dinucleotide phosphate (NADPH) to their substrate (Figure 4). This process converts drugs or chemical compounds to water-soluble compounds leading to be easily excreted in urine or feces (Williams et al., 2000).

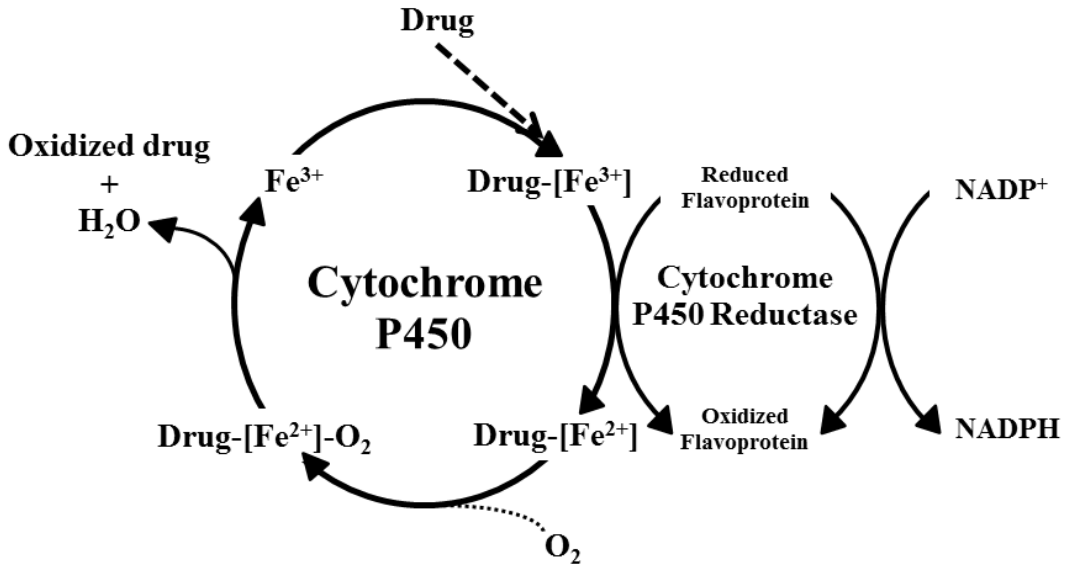

Figure 4 Monooxygenase reaction of xenobiotics by CYPs (Williams et al., 2000)

The function of most CYP enzymes is to catalyze the oxidation of organic substances. The substrates of CYPs include endogenous compounds, metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs, environmental pollutants, dietary chemicals, and other toxic chemicals. CYPs are the major enzymes involved in drug metabolism and bioactivation, accounting for ~75% of the total number of different metabolic reactions (Guengerich, 1988; Guengerich, 2008).

The most common reaction catalyzed by CYPs is a mono-oxygenase reaction, e.g., insertion of one atom of oxygen into an organic substrate (RH) while the other oxygen atom is reduced to water (Guengerich, 2008).

$$RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$$

The CYPs use a variety of small and large molecules as substrates in enzymatic reactions. Often, they form part of multi-component electron transfer chains, called CYP-containing systems. CYPs have been named on the basis of their cellular (cyto) location and spectrophotometric characteristics (chrome): when the reduced heme iron forms an adduct with carbonmonoxide (CO), CYP enzymes absorb light at wavelengths near 450 nm, identifiable as a characteristic Soret peak (Sigel

et al., 2007). CYPs enzymes have been identified in all domains of life, in animals, plants, fungi, protists, bacteria, archaea, and even viruses (Sigel et al., 2007; Danielson, 2002). In the present, more than 11,500 distinct CYP proteins are known (Nelson, 2009).

The developments of knowledge about CYPs have occurred over the past 20 years. One is certainly the completion of the human genome project, which set the number of human CYP genes at 57 isoforms (Table 1) (Guengerich, 2008). Human cytochrome P450s are highly abundant in the liver, in which the major isoforms include CYP1A2 (13%), CYP2C9 and CYP2C19 (20%), CYP2E1 (7%), CYP2A6 (4%), CYP2D6 (2%) and CYP3A4 (30%) (Shimada et al., 1994).

Table 1 Classification of human CYPs based on the major substrates

Sterols	Xenobiotics	Fatty acid	Eicosanoids	Vitamins	Unknown
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24A1	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17A1	2C18				4F22
19A1	2C19				4V2
21A2	2D6				4X1
27A1	2E1				4Z1
39A1	2F1				20A1
46A1	3A4				27C1
51A1	3A5				
	3A7				

CYPs are the major enzymes involved in drug metabolism, accounting for 75% (Figure 5A). Of the 57 human CYPs, five isoforms including CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 are involved in 95% of the reactions of marketed-drugs (Figure 5B) (Williams et al., 2004).

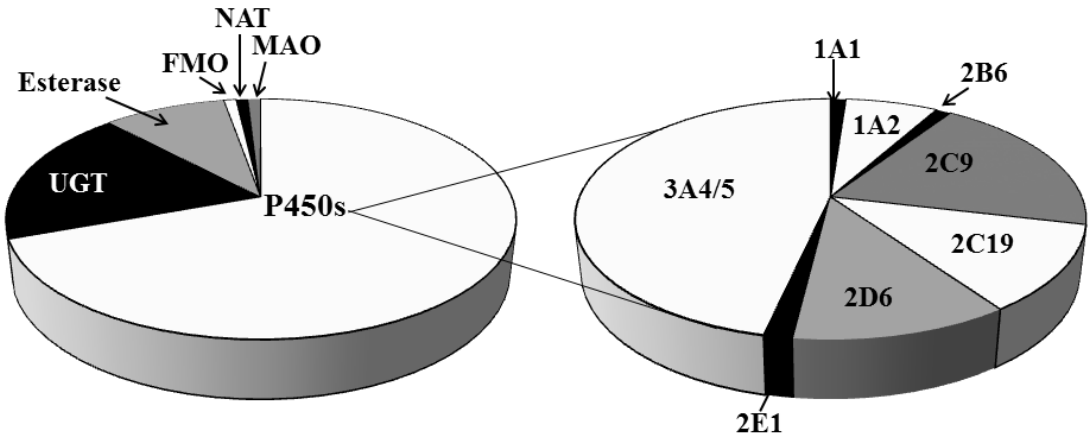

Figure 5 Contributions of drug-metabolizing enzymes involved in metabolism of marketed drug (Williams et al., 2004; Guengerich, 2008)

Deficiency of a particular CYP or that CYP is inhibited by another drug, the toxicity may develop, especially if drug accumulation occurs upon administration of multiple doses. Drug-drug interactions are recognized to be a major cause of adverse drug reactions (Guengerich, 2008; Guengerich et al., 2005). Normally, terfenadine is oxidized very rapidly by CYP3A4, and the major metabolite (fexofenadine) is responsible for the pharmacological activity (a tert-butyl methyl group is oxidized to a carboxylic acid). In individuals who used drugs that inhibited CYP3A4 such as ketoconazole or erythromycin, terfenadine was accumulated in the plasma and cardiac tissue. Moreover, dietary constituents, i.e., grapefruit, also inhibits CYPs, although not to the extent to present serious danger with terfenadine (Rau et al., 1997). Another example of toxicity from a parent drug is the anticoagulant warfarin, which has a relatively narrow therapeutic window (little interval in dose between being effective and being toxic in different individuals). Too low level of warfarin yields clotting, and too high the level increases a risk to hemorrhage.

The “effective dose” should be individually adjusted, and the optimal dose has been influenced by polymorphisms of CYP2C9 (Higashi et al., 2002).

Similar cases involve some environmental toxicants and carcinogens, although generally the parent compounds generate pharmacological activities of their own. However, one issue is metabolism (to harmless products) that will prevent distribution to tissues in which bioactivation may occur, thus preventing toxicity. For instance, metabolism in the liver can prevent distribution of polycyclic aromatic hydrocarbons (PAHs) to lung and other target tissues (Nebert, 1989). However, CYP1A1 is often considerably dangerous as this isoform activates PAHs (Uno et al., 2004). Therefore, effects of dietary supplements on regulatory mechanism of CYP1A1 expression should be strongly concerned.

2.2.2 Cytochrome P450 1A (CYP1A)

One of the most widely CYPs study is cytochrome P450 subfamily 1A (CYP1A) because CYP1A plays an important role in the metabolism of xenobiotic compounds such as halogenated aromatic hydrocarbons, PAHs, halogenated aromatic amines/amides, and dioxin. Therefore, CYP1A has been widely manipulated as a biomarker for exposure to carcinogen. CYP1A1 and CYP1A2, members of CYP1A subfamily, have been well-characterized in human and rodents (Yamazaki et al., 2000). CYP1A1 and CYP1A2 are involved in the activation of polycyclic arylamines, PAHs, and aflatoxin B1 (Gonzalez & Gelboin, 1994). PAHs are in turn inducers of CYP1A1 and CYP1A2 genes. Human CYP1A1 and CYP1A2 have overlapping substrate specificities (Shimada et al., 1996; Shimada et al., 1997). CYP1A1, and CYP1A2 are regulated in part by the Ah (Arylhydrocarbon) locus, but the mechanisms of transcriptional regulation of these two genes are not the same.

CYP1A1 expresses constitutively in several extrahepatic tissues, but poorly expresses in the liver. However, CYP1A1 expression has been demonstrated in the liver after inducer treatments such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3-methylcholanthrene (3-MC), and benzo[*a*]pyrene (B[*a*]P) (Okey, 1990). CYP1A1 variants and cancer risk have been investigated in several studies (Jacquet et al., 1996; Bartsch et al., 2000; Chi et al., 2009). Polymorphism of CYP1A1 was involved in the activation and detoxification of chemical carcinogens found in

tobacco smoke, it may influence host susceptibility to lung carcinoma (Bartsch et al., 2000; Hayashi et al., 1991). Moreover, CYP1A1 supports aryl hydrocarbons hydroxylase activity and a high activity has been associated with higher lung cancer risk (Jacquet et al., 1996; Stucker et al., 2000). CYP1A1 was demonstrated that individuals with a high level of CYP1A1 activity and gene expression were at greater risk for smoking-related cancers (Goth-Goldstein et al., 2000). CYP1A1 has been shown to be over-expressed in tumors and metabolizes procarcinogens to epoxide intermediates, which are further activated to diol epoxides by epoxide hydrolase (Murray et al., 1995; Shimada & Fujii-Kuriyama, 2004). Furthermore, CYP1A1 polymorphisms were associated in the Japanese population with tobacco smoke induced lung cancers (Nakachi et al., 1993; Nerurkar et al., 2000). Not only in Japanese, but also Chilean people carrying single CYP1A1 polymorphisms could be more susceptible to lung cancer induced by environmental pollutants such as PAHs (Quinones et al., 2001). However, the CYP1A1^{Ile462Val} polymorphism in never-smoking Korean women is associated with a low risk of lung adenocarcinoma, whereas specific combinations of variant genotypes for metabolic enzymes (such as CYP1B1^{Leu432Val}, GSTP1^{Ile105Val}) considerably increased the risk (Yoon et al., 2008). CYP1A1 was inducible by cigarette smoke condensate, subsequently increase risk of oral squamous cell carcinoma (Nagaraj et al., 2006). Therefore, the inducible expression of CYP1A and constitutive expression of CYP1A are considered to be an important marker of carcinogenesis.

Chi et al. (2009) revealed that B[*a*]P and tobacco induced CYP1A1 expression in oral squamous cell carcinoma (SCC) and oral mucosa.

The inter-individual variation in CYP1A1 was observed among smokers may account in part for variation in tobacco-related oral SCC risk (Chi et al. 2009). The most common chemical, extensively studied for its carcinogenicity, is B[*a]*P. Formation of B[*a*]P-7,8-diol-9,10-epoxides, referred to as bay region epoxides, causes acceleration of DNA mutations due to the high reactivity of these chemicals. A compound that interferes with this process, by blocking the formation of reactive intermediates, can potentially prevent the initiation of carcinogenesis (Ciolino et al., 1998). On the other hand, a compound, which can induce the expression of CYP1A1, might increase risk of carcinogenesis via CYP1A1 regulatory pathway.

CYP1A2 constitutively and inducibly expresses only in the liver (Kimura et al., 1986; Iwanari et al., 2002). Moreover, CYP1A2 is the key enzyme in the metabolic activation of heterocyclic amine to DNA binding form (Eisenbrand et al., 1993). The most potent carcinogen among all aflatoxins, aflatoxin B1 (AFB1), is considered to be one of potent carcinogen for hepatic cancer. Oxidation of the 8,9-vinyl bond of AFB1 results in biologically active AFB1-8,9-epoxide, which reacts with DNA, RNA, and proteins. AFB1 metabolism results in several hydroxylated metabolites (Eaton et al., 1994). Both activation and deactivation of AFB1 are mediated by the microsomal CYP system, by which CYP1A2 is a major metabolizing enzyme involved in both epoxidation and hydroxylation of AFB1 in human liver. CYP1A2 has greater affinity for AFB1, and AFB1 is therefore considered to be more active in the metabolism (Gallagher et al., 1996). There was the evidence showed high activity of CYP1A2 increased risk of lung cancer (Seow et al., 2001).

2.2.3 Regulatory pathways of CYP1A

CYP1A1 regulation is initiated by at least three different signaling pathways. The first one, which is certainly well documented, is the arylhydrocarbon receptor (AhR) signaling pathway. The second one involves the retinoic acid receptors (RARs), but remains yet to be confirmed. Finally, the third one is tyrosine kinases pathway, but still needs to be thoroughly investigated (Delescluse et al., 2000).

A ligand-activated transcription factor, AhR, is a basic Helix-Loop Helix protein belonging to the Per-Ah receptor nuclear translocator (Arnt)-Sim (PAS) family. It is a cytosolic transcriptional factor that is normally inactive, bound to several co-chaperones. Non-ligand bound AhR is retained in cytoplasm as an inactive protein complex consisting of a dimer of 90 kDa heat shock protein (Hsp90). Hsp90 is a chaperone protein system that prevents transcriptional activation of the AhR but constraining the receptor in a form that facilitates ligand binding. Hsp90 also protects AhR from proteolysis (Delescluse et al., 2000; Nebert et al., 2004). AhR is present in the cytosol as a multi-protein complex containing two molecules of hsp90 (Ma & Whitlock, 1997). When AhR ligand, lipophilic ligands such as dioxin and PAHs, crosses the plasma cell membrane, ligand will bind to the AhR. After ligand binding,

a dimer of Hsp90 is released and the ligand bound AhR is translocated into the nucleus and binds to its partner, designated as the Ah receptor nuclear translocator (Arnt) (Figure 6). This heterodimer of AhR and Arnt interacts with 5'-GCGTG-3' DNA sequence, the core binding motif of the xenobiotic responsible element (XRE). Binding of heterodimer of AhR:Arnt and XRE leads to changes in the chromatin structure allowing transactivation of AhR-controlled genes. After transcription occurred, mRNA will be sent to ribosome for translation process. CYP1A1 protein will be produced, and causes toxic response. Halogenated aromatic hydrocarbons and PAHs including dioxins are known to bind to the AhR as exogenous ligands, usually causing toxicological effects through AhR-dependent biological changes (Fernandez-Salguero et al., 1996).

Following ligand binding in the cytoplasm, AhR is internalized and dimerized with Arnt. This heterodimer acquires the ability to interact with XRE and enhances transcription of CYP1A1 gene. Moreover, low dose of retinoic acid (RA), a natural vitamin A metabolite, slightly induces cutaneous CYP1A1 expression by activation of the nuclear receptor, retinoid acid receptors (RAR). The RAR-RXR (retinoid X receptor) heterodimer binds the RA responsive element sequence located within the CYP1A1 promoter (Vecchini et al., 1994). Many carotenoids (α- and β-carotene, β-cryptoxanthin), in part contained in food, can be metabolized into products with retinoid activity and activate CYP1A1 through this signaling pathway (Delescluse et al., 2000).

As we know, most of the effects of a popular dioxin, TCDD, has been attributed to a mechanism by which TCDD binds to the AhR. However, this mechanism does not able to account for some dioxin effects that occur rapidly, such as the increased calcium (Ca^{2+}) uptake, protein tyrosine kinase (PTK) activation and the non-transcriptional regulation of proteins (Delescluse et al., 2000).

Moreover, numerous studies have shown that many of TCDD's effects result from changes in cell growth and differentiation. Because protein kinases (PKs), particularly PTKs, are known to play such an important role in regulating cell growth and differentiation, extensive researches have been done on the potential role of PKs in the mechanism of TCDD action. PKs activation is one of the most consistently observed biochemical effects of TCDD; it occurs in many species, many tissues and

cell culture systems. Such an activation has clearly been demonstrated to be dependent on TCDD binding to the AhR as shown by the dose-response and structure-activity relationships, as well as the use of responsive and non-responsive strains of animals or the use of AhR blockers. Thus, the ability of TCDD to affect PKs activity, particularly PTKs, including the phosphoprotein (60 kD) encoded by the Srconcogene ($pp60^{Src}$) has been clearly shown to be an important step in the mechanism of action of TCDD (Blankenship & Matsumara, 1997; Kohle et al., 1999).

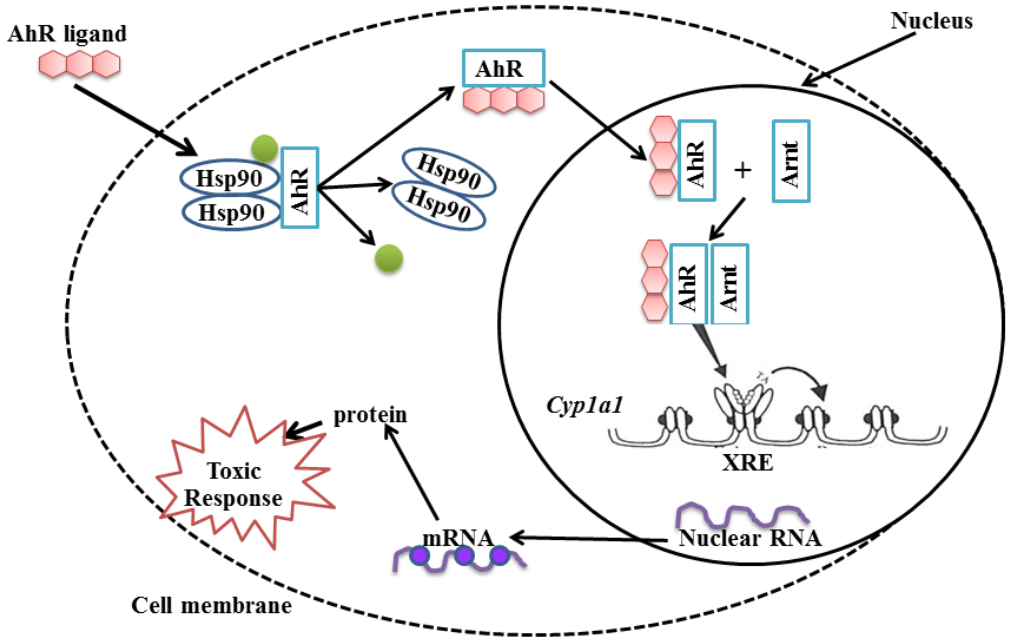

Figure 6 Aryl hydrocarbon receptor signaling pathway (Delescluse et al., 2000)

All these results are consistent with the idea that the AhR and a PTK coexist in the cytosol, as part of a multimeric protein complex that can be specifically co-immunoprecipited. In fact, it is not uncommon to find kinases and in particular $pp60^{Src}$ associated with hormone receptors. The complex formed between AhR, Hsp90 and $pp60^{Src}$, would dissociate into the nucleus instead of the cytoplasm. AhR heterodimerized with Arnt, Hsp90 and $pp60^{Src}$, plays the role of facilitating and amplifying the function of the receptor to transduce the message. They could also participate in inter-relationships between the numerous intracellular receptors (RAR: retinoic acid receptor; GR: glucocorticoid receptor; ER: estrogen receptor; PR:

progesterone receptor) and signaling pathways (Figure 7) (Blankenship & Matsumara, 1997; Delescluse et al., 2000).

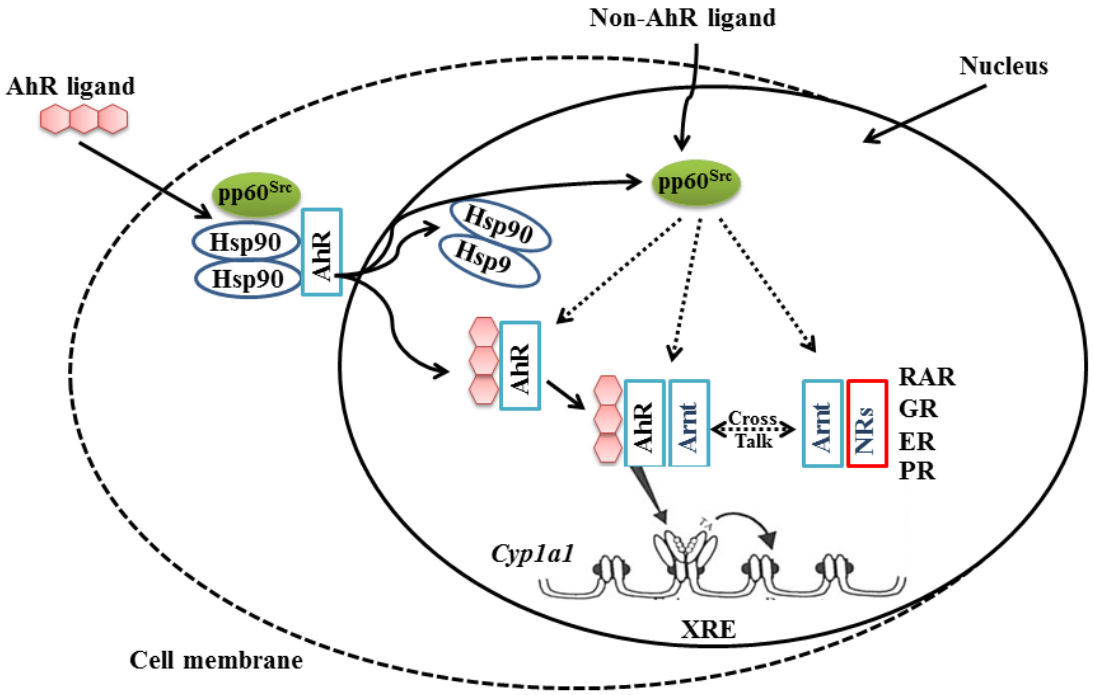

Figure 7 The induction of CYP1A1 transcription via AhR, Hsp90, pp60^{Src}, and RAR (Blankenship & Matsumara, 1997)

Recently, regulatory pathway of human CYP1A1 and CYP1A2 via constitutive androstane receptor (CAR) was reported (Yoshinari et al., 2010). CAR, a member of nuclear receptor superfamily, plays a key role in the drug-induced expression of CYP2A, CYP2B, CYP2C, and CYP3A enzymes, combines with pregnane X receptor (PXR) (Sueyoshi & Negishi, 2001). CAR is normally retained in cytoplasm and translocates to the nucleus in response to the exposure of drugs such as phenobarbital (PB). In nucleus, CAR heterodimerizes with retinoid X receptor α (RXRα), binds to *cis*-elements in the promoter region of target genes, and enhances the transcription. Involvement of CAR in the drug-induced expression of CYP1A1 and CYP1A2 is also suggested. Treatment with PB resulted in the increased hepatic CYP1A2 mRNA levels in AhR-null mice (Zaher et al., 1998) and DBA/2 mouse which is an AhR-nonresponsive strain (Sakuma et al., 1999). These studies suggested that increases of CYP1A1 and CYP1A2 expressions possibly through a pathway

independent of AhR. Human CAR (hCAR) enhanced the transcription of both CYP1A1 and CYP1A2 genes through common promoter regions from -461 to -554 and from -18089 to -21975 of CYP1A1. An everted repeat separated by eight nucleotides (ER8) motif was located at around -520 of CYP1A1, and ER8 was identified as an hCAR-responsive element and a binding motif of hCAR/human RXR heterodimer (Yoshinari et al., 2010). CAR transactivates human CYP1A1 and CYP1A2 in human hepatocytes through the common *cis*-element ER8. Interestingly, the ER8 motif is highly conserved in the CYP1A1 proximal promoter sequences of various species, suggesting a fundamental role of CAR in the xenobiotic-induced expression of CYP1A1 and CYP1A2 independent of aryl hydrocarbon receptor (Figure 8) (Yoshinari et al., 2010).

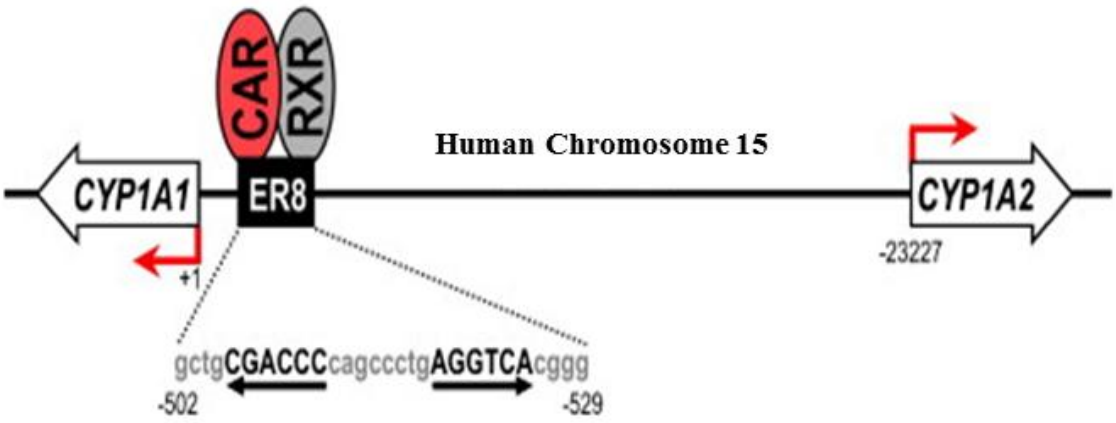

Figure 8 CAR transactivates human CYP1A1 and CYP1A2 through the common *cis*-element ER8 (Yoshinari et al., 2010)

2.2.4 Effect of *A. paniculata* and andrographolide on cytochrome P450s

Previous study reported that *A. paniculata* induced mouse hepatic CYP1A1 and CYP2B via significant increases of ethoxyresorufin *O*-dealkylase (EROD) and pentoxyresorufin *O*-dealkylase activities (Jarukamjorn et al., 2006). CYP1A2 mRNA and EROD activities were inhibited by andrographolide in human hepatocytes and the inhibition was categorized as noncompetitive inhibition of EROD metabolism by CYP1A2 using a Lineweaver-Burk plot (Pekthong et al., 2008).

A. paniculata leaf extract and andrographolide inhibited hepatic microsomal CYP2E1 via inhibition of aniline hydroxylase and *O*-demethylase of *p*-nitroanisole in rats (Choudhury et al., 1987). The ethanolic extract of *A. paniculata* exhibited mixed type inhibition of CYP2C19 enzyme activity *in vitro* (Pan et al., 2011a). *A. paniculata* aqueous and hexane extracts, and andrographolide showed weak inhibition of CYP2C9-mediated tolbutamide 4-hydroxylation, but the ethanolic and methanolic extract potentially inhibited CYP3A4-dependent testosterone 6β-hydroxylation and CYP2C9-dependent tolbutamide methylhydroxylation activities (Pan et al., 2011b). Moreover, andrographolide significantly induced the expression of CYP1A1 mRNA in a concentration-dependent manner in mouse hepatocytes in primary culture, as did typical CYP1A inducers. Interestingly, andrographolide plus a typical CYP1A inducer synergistically induced CYP1A1 expression, and the synergism was blocked by an arylhydrocarbon receptor (AhR)-antagonist, resveratrol (Jaruchotikamol et al., 2007). Recently, Pekthong et al. (2009) reported the effect of *A. paniculata* extract or andrographolide on the expression of a limited number of cytochrome P450 *in vivo* in rat liver and *in vitro* in human and rat hepatocyte cultures, indicating that the effect was diversed. For example, the treatment decreased expression of human CYP2C9 and CYP3A4 or rat CYP2C11 and CYP3A1, while CYP1A2 expression was enhanced in rats. *A. paniculata* extract was confirmed to inhibit CYP2C9, CYP2D6, and CYP3A4 enzyme activities by luminescent assay in recombinant human cytochrome P450 2C9, 2D6 and 3A4 enzymes (Hanapi et al., 2010). Very recently, andrographolide and 14-deoxy-11, 12-didehydroandrographolide were reported to be potential inhibitor of CYP3A4 in HepG2 hepatoma cells (Ooi et al., 2011), by which 14-deoxy-11, 12-didehydroandrographolide inhibited CYP3A4 by binding and antagonizing PXR function (Ooi et al., 2011).

2.2.5 Effect of andrographolide on level of glutathione

Glutathione *S*-transferase (GST) is a phase II enzyme that catalyzes the conjugation of glutathione (GSH) with a variety of electrophilic xenobiotics. GST is divided into cytosolic, mitochondrial, and microsomal families. The π class of GST (GSTP) belongs to the cytosolic class (Hayes et al., 2005). GSTP is not expressed in healthy liver, but it is dramatically and specifically increased in both chemically

induced and spontaneously arising precancerous lesions and hepatomas in experimental carcinogenesis studies (Farber, 1984; Sato et al., 1989). The study of Chang et al. (2008) showed that ethanolic and ethyl acetate extracts of *A. paniculata* and andrographolide dose-dependently induced GSTP protein and mRNA expression in primary rat hepatocytes. The activity of GST was also increased in the hepatocytes treated with the extracts of *A. paniculata* and andrographolide. In the presence of andrographolide, level of reduced glutathione (GSH) in HepG2 cell was decreased (Li et al., 2007). Another report of Lili et al. (2009), showed that andrographolide initially increased intracellular GSH levels which then decreased later in hepatoma Hep3B cells. Furthermore, pretreatment with andrographolide protected the cardiomyocytes against hypoxia/reoxygenation injury and up-regulated the cellular GSH level (Woo et al., 2008). The intracellular level of GSH in HepG2 cells was significantly decreased after the addition of andrographolide (Zhang et al., 2008). When the molecular interaction between andrographolide and GSH was investigated under a condition mimicking the *in vivo* environment, the level of GSH dropped in the presence of andrographolide (Zhang et al., 2008). These evidences show that *A. paniculata* extracts and andrographolide have marked effects on hepatic biotransformation both phase I and II.