CHAPTER II

LITERATURE REVIEWS

Curcuma comosa Roxb.

Curcuma comasa Roxb. (Figure 1) is a medicinal plant in family Zingiberaceae. Its is commonly known as Waan chak mod look, which is refered to its involuting action on uterus. Plants of the Curcuma species including, *C. aromatica* Salisb., *C. comosa* Roxb., *C. longa* Linn., *C. parviflora* Wall., *C. xanthorriza* Roxb. and *C. zedoaria* Roxb (วงศ์สถิตย์ ฉั่วสกุล, 2533). They have been used as indigenous medicines for treatment of a wide range of disease in many Asian countries, such as China, India, Japan, Indonesia, Malaysia, and Thailand. In Thailand, the rhizome of this plant has been widely used as a traditional medicine in many provinces particularly in the Eastern part of the country along Cambodia border and North-eastern part of Thailand (สมพร หิรัญรามเดช และ เจมส์ แฟรงคลิน แมกซ์เวล, 2534, 2535). Its rhizome is used for treatment of the inflammation of postpartum uterine bleeding. From the previous investigations, the hexane extract of *C. comosa* was demonstrated to possess a weak estrogenic-like activity and mediated its action through specific estradiol nuclear binding receptor (Piyachaturawat *et al.*, 1995b). Plants in Curcuma species including *C. comosa* have been reported to contain a number of diarylheptanoids (Suksamrarn *et al.*, 1997; Cleason *et al.*, 1993, 1994). Biological activities of diarylheptanoids are mostly reported to have anti-inflammatory and anti-hepatotoxic effects (Claesos *et al.*, 1994). Several of diarylheptanoids have been demonstrated to inhibit prostaglandin and leukotriene biosynthesis in vitro (Kiuchi *et al.*, 1982; Flynn *et al.*, 1986; Kiuchi *et al.*, 1992; Ammon *et al.*, 1992). The study on structure-activity relationship of antihepatotoxic effect of diarylheptanoids suggested that free hydroxyl groups in aryl moieties were a major structural requirement for potent antihepatotoxic effect (Hikino *et al.*, 1985).

Natural compounds found in rhizome of *C. comosa* can be classified on the basis of their chemical structures as following: (Suksamrarn *et al.*, 1994, 1997; Piyachaturawat *et al.*, 2000, 2002).

1 Diarylheptanoids: *trans*-1,7-diphenyl-5-hydroxy-1-heptene, *trans*-1,7-Diphenyl-6-hepten-3-one-5-ol, *trans*-1,7-diphenyl-3-acetoxy-6-heptene, *trans*-1,7-diphenyl-6-heptene-3-one, *trans,trans*-1,7-diphenyl-1,3-heptadien-5-ol, *trans,trans*-1,7-diphenyl-4,6-heptadien-3-one, 1,7-diphenyl-(*1E,3E,5E*)-heptatriene, 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(*1E*)-1-heptene), 7-(3,4-dihydroxyphenyl-5-hydroxy-1-phenyl-(*1E*)-1-heptene.

2. Acetophenones: 4,6-dihydroxy-2-O-(beta-D-glucopyranosyl) acetophenone.

The chemical structures of these following compounds were shown in Table 1.

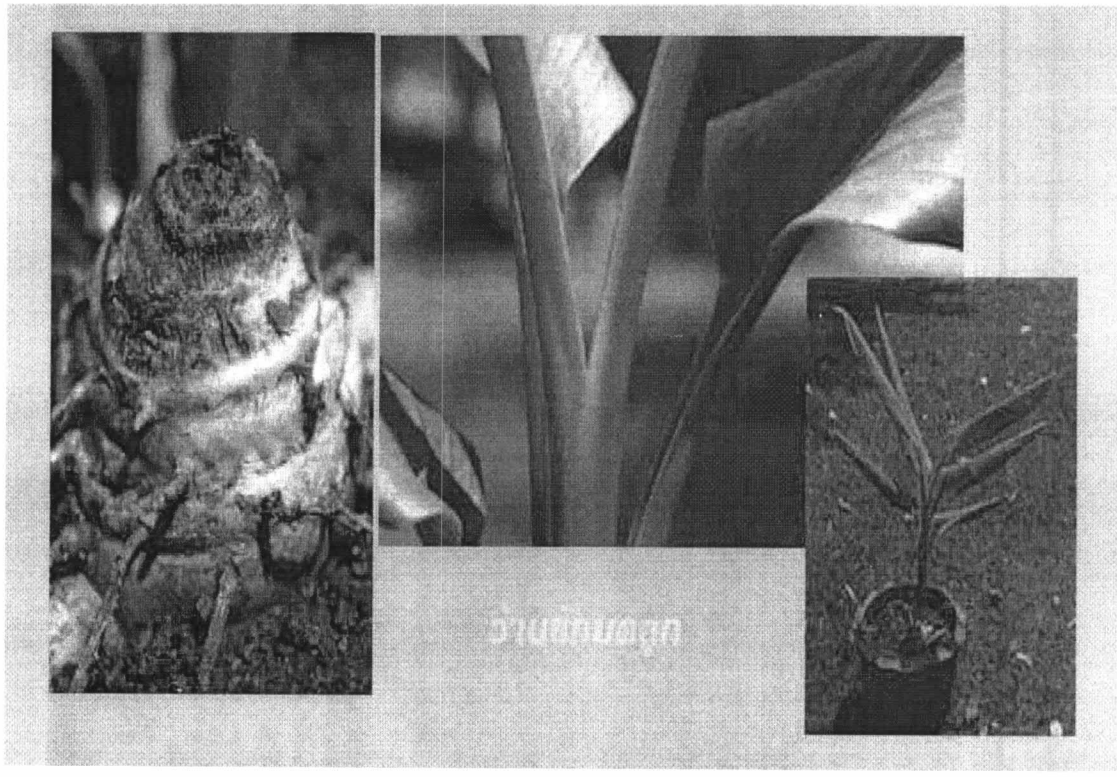


Figure 1 *Curcuma comosa* Roxb. plant

Table 1 Chemical structures of compounds found in rhizome of *C. comosa*

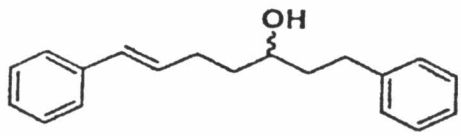
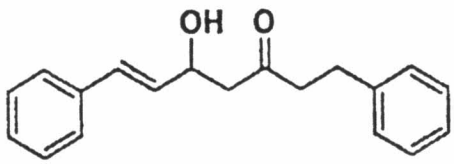
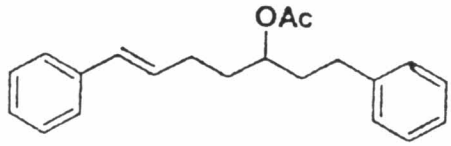
Compound	Extraction fraction	Bioactivity	References
1.Diarylheptanoids  *trans*-1,7-Diphenyl-5-hydroxy-1-heptene	hexane CH_3OH	Anti-inflammatory activity Nematocidal activity	Claeson et al., 1993 Jurgen et al., 1994
 *trans*-1,7-Diphenyl-6-hepten-3-one-5-ol	CH_3OH	Nematocidal activity	Jurgen et al., 1994
 *trans*-1,7-Diphenyl-3-acetoxy-6-heptene	CH_3OH	Nematocidal activity	Jurgen et al., 1994

Table 1 (Con't) Chemical structures of compounds found in rhizome of *C. comosa*

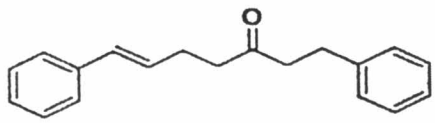
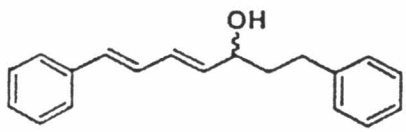
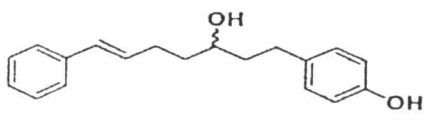
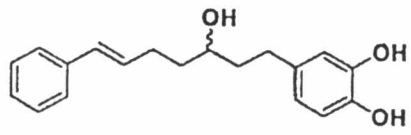
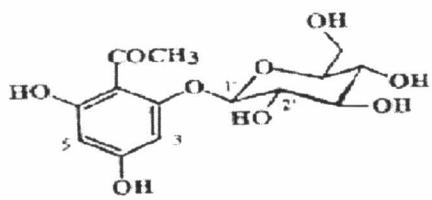
Compound	Extraction fraction	Bioactivity	References
 *trans*-1,7-Diphenyl-6-heptene-3-one	CH_3OH	Nematocidal activity	Jurgen et al., 1994
 *trans,trans*-1,7-Diphenyl-1,3-heptadien-5-ol	hexane CH_3OH	Anti-inflammatory activity Nematocidal activity	Claeson et al., 1993 Jurgen et al., 1994
 5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(*1E*)-1heptene	EtOAc	Hypolipidemic activity Choleretic activity	Suksamrarn et al., 1994 Suksamrarn et al., 1997

Table 1 (Con't) Chemical structures of compounds found in rhizome of *C. comosa*

Compound	Extraction fraction	Bioactivity	References
 7-(3,4-Dihydroxyphenyl)-5-hydroxy-1-phenyl-(*1E*)-1-heptene	EtOAc	Choleretic activity	Suksamrarn et al., 1997
 4,6-dihydroxy-2-*O*-(*β*-D-glucopyranosyl) acetophenone	EtOAc	Choleretic activity	Suksamrarn et al., 1997

Pharmacological effects

1. Uterotrophic effects

Uterotrophic activities of all four crude extracts (hexane, ethyl acetate, butanol and aqueous extracts) of *C. comosa* were studied in rats. It was found that the hexane extract was most effective in increasing of uterine weight, glycogen content and the cornification of vaginal epithelium as well as the keratinization of mucosal surface of vagina. Butanol and ethyl acetate extracts produced slight increases, whereas the aqueous extracts had no effects on the same parameters mentioned above (Piyachaturawat *et al.*, 1995a). In the same year, Piyachaturawat *et al.* (1995b) investigated the estrogenic activity of the hexane extract of *C. comosa* by administrating intraperitoneally at a dose of 480 mg/kg for two consecutive days to mature ovariectomized rats. The results showed that the extract significantly increased vaginal mucosa thickness and uterine wet weight. Histology of the vaginal mucosa in the *C. comosa* treated animals showed a marked proliferation and keratinization similar to those in the estradiol treated animals. It was found that most effect of this extract corresponded to the specific estrogenic action. The extract effectively induced an increase of specific estradiol binding site in the uterine nuclei.

Effects of 95% ethanolic extract from rhizome of *C. comosa* on the contraction of intact and isolate rat uterus were investigated. The results of isolated rat uterus showed that both 5 and 10 μg/ml of this extract reduced contraction induced by oxytocin, acetylcholine (Ach), serotonin (5-HT) and potassium chloride (KCl) in DE Jalon solution and 10 μg/ml of this extract also reduced contraction induced by oxytocin, vanadate and $PGF_{2\alpha}$ in Ca^{2+}-free Lock Ringer solution with ethylene glycol bis (β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA). In anesthetized rat, intraperitoneal injection of 0.5 g/kg of this extract also markedly reduced uterine contraction stimulated by oxytocin (อนุกูล สวัสดิ์พาณิชย์, 2537).

2. Other estrogenic-like action

The growth suppressing activity of *C. comosa* extract on male reproductive organs was investigated in immature rats. Intragastric administration of the hexane extract at the dose of 500 mg/kg body weight for 7 consecutive days significantly suppressed weights of testes, epididymis, ventral prostrate, seminal vesicle and levator ani muscle whereas the same dose of ethyl acetate extract did not affect the organ weights. Histological examination revealed regression of the spermatogonium in seminiferous tubules and necrosis of epithelial cells in the epididymis (Piyachaturawat *et al.*, 1998). These effects had been confirmed by the same authors. It was found that the hexane extract of *C. comosa* was able to decrease testicular weight corresponded with a marked regression of spermatogonia and spermatids in the seminiferous tubules in adult rats (Piyachaturawat *et al.*, 1999).

3. Choleretic effect

In 1996, Piyachaturawat and collaborates demonstrated that all four crude extracts of *C. comosa* including hexane, ethyl acetate, butanol and aqueous extracts possessed choleretic properties. An intraduodenal injection (1.0 g/kg body weight of the extract) into rats increased bile flow rate. The butanol extract was the most potent choleresis with increased bile flow rate (BFR) and followed by ethyl acetate, hexane and aqueous extracts, respectively. The choleresis of ethyl acetate extract was found to be dose-dependented. For other biliary constituents, both butanol and ethyl acetate extracts (1 g/kg) as well as ethyl acetate fraction markedly increased the concentrations and outputs of bilirubin, cholesterol and calcium. However, the greatest effects were produced by the ethyl acetate and butanol extracts.

In 1997, Suksamrarn and collaborates demonstrated that the ethyl acetate and butanol extracts of *C. comosa* were found to exhibit choleretic activity in rats. It was found that the diarylheptanoids compound (i.e. 1,7-diphenyl-5-hydroxy-(1E)-1-heptene, 5-hydroxy-7-(4-hydroxyphenyl)-1-phenyl-(1E)-1-heptene and 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-(1E)-1-heptene), were isolated from the ethyl acetate extract of

C. comosa rhizomes. A phloracetophenone glucoside, 4,6-dihydroxy-2-O-(β-D-glucopyranosyl)acetophenone, was isolated from the ethyl acetate and n-butanol extracts.

In 1998, Piyachaturawat and collaborates investigated the effects of synthetic phloracetophenone (2,4,6-trihydroxyacetophenone, THA) on bile flow and biliary lipid secretion in male rats. A single intraduodenal administration of THA at the dose of 10-150 mg/kg induced a dose-dependent increase of bile flow rate. The increase of bile flow was associated with increased biliary secretion of bile acid, decreased secretion of cholesterol, phospholipid, and lowered bile lithogenic index. THA at a dose of 100 mg/kg body weight induced a maximal increase of bile flow rate and bile acid output.

In 2002, Piyachaturawat and collaborates investigated the choleretic effects of THA in hypercholesterolemic male hamsters. Intragastric administration of THA (300-600 μmol/kg) twice daily for 7 days to these animals caused a dose- and time-dependent decreases in both plasma cholesterol and triglyceride levels. THA at a dose of 400 μmol/kg reduced cholesterol and triglyceride levels in plasma with decreases in both plasma very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol but not in high density lipoprotein (HDL) cholesterol.

4. Hypolipidemic effect

In 1997, Piyachaturawat and collaborates investigated the hypolipidemic activity of the ethyl acetate extract of *C. comosa* in mice. It was found that this extract was able to decrease plasma lipid levels of both triglyceride and cholesterol but increased liver triglyceride content.

Intragastric administration of the ethyl acetate extract of *C. comosa* rhizome (0-500 mg/kg body weight per day) to hypercholesterolaemic hamsters for 7 days decreased both plasma triglyceride and cholesterol levels in a dose-dependent manner.

The *C. comosa* extract also increased plasma HDL-cholesterol and decreased plasma LDL-cholesterol.

5. Nematocidal effect

Hexane-soluble fraction of the methanol extract of *C. comosa* had nematocidal activity. It was found that *trans*-1,7-diphenyl-5-hydroxy-1-heptene was the most potent inhibitor of nematode mortality with an EC_{95} of 0.7 μg/ml. *trans*-1,7-Diphenyl-6-hepten-3-one-5-ol was slightly less active (1μg/ml), followed by *trans*-1,7-diphenyl-3-acetoxy-6-heptene (9 μg/ml) and *trans*-1,7-diphenyl-6-hepten-3-on (9 μg/ml), which were less potent, and finally *trans,trans*-1,7-diphenyl-1,3-hepatien-5-ol, which was inactive (>100 μg/ml) (Jurgens *et al.*, 1994).

Toxicological effects

1. Subacute toxicity

Toxicity of the choleretic compound, THA was investigated in mice, rats and hamsters. Acute toxicity of THA was observed to be dependent on species and route of administration, but not sex and age. Median lethal dose (LD_{50}) of THA given intraperitoneally to adult male hamsters and mice were 338 and 365 mg/kg body weight, respectively. It was significantly increased to 489 mg/kg body weight in adult male rats and greatly increased by intragastric (i.g.) route. Subacute toxicity was investigated in adult male rats by giving THA at doses of 37-300 mg/kg body weight/day, i.g. for 30 consecutive days. High doses of THA induced periportal hepatocyte degeneration whereas plasma concentrations of alanine aminotransferases, aspartate aminotransferases, bilirubin, blood urea nitrogen, and hepatic triglyceride content were only slightly increased (Piyachaturawat et al., 2002).

2. Subchronic toxicity

Ethanolic extract of *C. comosa* rhizome was investigated in subchronic toxicity study. The extract was intragastric administered to six groups of Wistar rats for 90 consecutive days. The experimental groups were given the extract at doses of 100, 200, 400 and 800 mg/kg/day, respectively while two control groups received distilled water and solvent vehicles, respectively. The extract did not affect growth and food consumption of rats. Decreasing of hematocrit and hemoglobin in male rats receiving the highest dose of the extract were still within the normal range. Male rats treated with 800 mg/kg and female rats receiving 400 and 800 mg/kg of the extract had a significant increase of alkaline phosphatase level. Increases in stomach weight and size were observed in male rats receiving the extract at a dose of 100 mg/kg or more and in females at a dose of 200 mg/kg and more. Histopathological examination of visceral organs revealed no abnormality related to the extract except hyperplasia and hyperkeratosis of the gastric epithelium, the effects of which were dose-dependent (Chivapat et al., 2003).

Phytoestrogens

Phytoestrogens are estrogenic compounds found in plants. They are found in many human foodstuffs that are part of daily intake (Ganry, 2004). Some of them also possess anti-estrogenic actions in mammalian tissues (Kenneth, 1998). Phytoestrogens are a diverse group of compounds with regard to their chemical structures as well as their biological activities. Based on the chemical structure of non-steroidal phytoestrogens, there are three main classes: 1) flavonoids (flavones, isoflavones, and chalcones) such as genistein, kaemferol, myricetin, and apigenin; 2) coumestans; and 3) mammalian lignans (Murkies *et al.*, 1998; Institute of food science, 2001; Ganry, 2004; Cornwell *et al.*, 2004).

Phytoestrogens and disease relationships

1. Bone density

Estrogens play an important role in maintaining bone density by regulating the formation and resorption of bone (Nilsson and Gustafsson, 2002). Most of the studies suggest that phytoestrogens are somewhat effective in maintaining bone mineral density (BMD) in postmenopausal women (Dalais *et al.*, 1998; Kardinaal *et al.*, 1998; Alekel *et al.*, 2000; Chiechi *et al.*, 2002; Kim *et al.*, 2002; Morabito *et al.*, 2002). A double blind placebo controlled study was performed in postmenopausal women. The results showed that there was significant increase in BMD at the femoral necks after 12 months of daily admistration of 54 mg genistein, isolated from soy. However a significant increase in osteocalcin and bone specific alkaline phosphatase (BAP) was also observed (Morabito *et al.*, 2002).

2. Cardiovascular diseases

Estrogen can affect vascular system both directly, through the estrogen receptors (ERs) located in vascular tissue and indirectly through altering the lipoprotein profile (Rubanyi *et al.*, 2002). There is an evidence to support that phytoestrogens may be cardioprotective. A study in Rhesus monkeys, using diets made from soy showed that cardioprotective of phytoestrogens is probably attributed to the hypocholesterolemic effect. This study showed a relatively large reduction in LDL-cholesterol, VLDL-cholesterol, and total cholesterol, as well as an increase in the ratio of HDL-cholesterol and LDL-cholesterol (Anthony *et al.*, 1995). There are several clinical studies examining the effect of dietary phytoestrogens on cardiovascular diseases (CVD). Isoflavonoids or soy/soy protein and flaxseed have the ability to lower total cholesterol (Potter *et al.*, 1998; Demlow *et al.*, 2000; Lucas *et al.*, 2002; Kaspers *et al.*, 2005), LDL-cholesterol (Wangen *et al.*, 2001; Jayagopal *et al.*, 2002; Lucas *et al.*, 2002) and to raise HDL-cholesterol (Potter *et al.*, 1998; Sanders *et al.*, 2002).

3. Cognitive function

Many postmenopausal women feel that their cognitive abilities decline during menopause, and some believe that hormone replacement therapy (HRT) may alleviate this decline. A few studies have examined effects of phytoestrogens on cognitive function. One study demonstrated that ten-week diets high in soy increased students' long term and short-term memory. Students in the high isoflavone diet group had significant improvements in short term and long term memory, and mental flexibility (File *et al.*, 2001). Duffy and collaborates (2003) compared the cognitive functions of postmenopausal women who took soy isoflavonoids to the postmenopausal women who received placebo. The woman receiving soy isoflavonoids showed an increase in recall of pictures, sustained attention, and ability to plan task.

4. Cancer

Genistein and daidzein have been extensively studied for anti-breast cancer and anti-prostate cancer because of their estrogenic and antiestrogenic activities. Genistein was shown to inhibit tyrosine specific protein kinases (Akiyama *et al.*, 1987), DNA topoisomerase II (Yamashita *et al.*, 1990), epidermal growth factor-induced phosphatidylinositol turnover (Imoto *et al.*, 1988) and angiogenesis (Fotsis *et al.*, 1993). Genistein has been more extensively studied and seems to be a more promising cancer protective agent than the closely related isoflavone and daidzein. Genistein has been shown to inhibit the growth of cancer cells through modulation of genes involed in the homeostatic control of the cell cycle and apoptosis. Genistein has been shown to inhibit activation of nuclear transcription factor, NF-kappa B and the Akt signaling pathway, both of which are known to maintain balance between cell survival and programmed cell death (apoptosis) (Sakar *et al.*, 2002; Fritz *et al.*, 2002).

5. Menopausal symptoms

Some women experience a decrease in the quality of life during menopause due to sleep deprivation, hot flashes, mood swings, forgetfulness and difficulty concentrating. However, a recent study by Women's Health Initiative (Hays *et al.*, 2003),

found relatively few benefits of HRT for quality of life parameters. A subset of woman, 50-54 years of age with moderate to severe symptoms, 56 mg of genistein had significant decreases in sleep disturbance and hot flashes (Hays *et al.*, 2003). Several studies showed a significant drop in the severity and frequency of menopausal symptoms compared with the beginning of the study (Clifton-Bligh *et al.*, 2001; Nikander *et al.*, 2003; Tice *et al.*, 2003).

Effect of phytoestrogens on hepatic drug metabolizing enzymes

Several previous studies have investigated the effects of flavonoids on drug metabolizing enzymes (Breinhott *et al.*, 2000; Robert-Kirchhoff *et al.*, 1999; Helsby *et al.*, 1998; Nielsen *et al.*, 1998; Zhai *et al.*, 1998; Helsby *et al.*, 1997; Siess *et al.*, 1995). A variety of flavonoids were shown to influence xenobiotic metabolism by induction of some CYP isoforms (i.e. CYP1A1, CYP1A2, CYP2B1&2B2) as well as phase II enzymes, GST and UDPGT in an *in vivo* study (Siess *et al.*, 1992). In contrast, inhibition effects of various flavonoids on those CYP isoforms (i.e. CYPs 1A1, 1A2, 2B1, 2B2 and 3A4) were demonstrated in the *in vitro* studies (Zhai *et al.*, 1998; Siess *et al.*, 1995; Li *et al.*, 1994). Isoflavonoids such as genistein, daidzein affected differently on hepatic drug metabolizing enzymes. Genistein and daidzein (or its metabolite, equol) demonstrated no induction effects on CYPs (i.e. CYPs 1A1, 1A2, 2B1&2B2, 2E1 and 3A) as well as the phase II enzymes, GST and UDPGT in the *in vivo* studies (Kishida *et al.*, 2000; Breinholt *et al.*, 1999, Helsby *et al.*, 1997). However, in an *in vitro* study, they inhibited CYP1A1&1A2 and CYP2E1. The inhibition effects of genistein and daidzein on CYP1A1 and CYP2E1 offered a possible explanation for their chemopreventive effects against chemical carcinogenesis (Helsby *et al.*, 1998).

Xenobiotic biotransformation

Xenobiotic metabolism is a biological process which alters many exogenous substances to terminal or alteration of biological activity. In general, lipophilic xenobiotics are transformed to more polar and hence more readily excreatable

products. The enzyme systems responsible for the biotransformation of many drugs are located in the smooth endoplasmic reticulum and cytosol of livers cell or hepatocytes. These enzymes are also found in other organs such as kidney, lung and gastrointestinal tract. The chemical reactions of enzymatic biotransformation are divided into phase I and phase II reactions. A summary of metabolic reactions is given in Table 2

Xenobiotics, after converted by specific enzymes to more reactive, more electrophilic intermediates, are capable of reacting covalently with biological macromolecules such as proteins, nucleic acids or lipids. Binding of xenobiotic metabolites to DNA may cause modification of genetic information, mutation and a consequent possibility of malignant growth (Soucek and Gut, 1992).

Phase I metabolism (or functionalisation reactions) includes oxidation, reduction, hydrolysis and hydration reactions, are mostly to introduce reactive functional groups on the molecule of the parent compounds, such as hydroxyl (-OH), primary amine ($-NH_2$), sulhydryl (-SH) and carboxyl (-COOH) groups. The reactions of phase I change many xenobiotics to the more polar metabolites or more hydrophilic and also provide functional groups that are used to complete phase II reactions. A variety of specific CYP isoforms, especially CYP in family 1, 2 and 3 are involved in the inactivation of certain chemical procarcinogens (Soucek and Gut, 1992; Parkinson, 2001).

Three CYP families CYP1, CYP 2, and CYP 3 are the major enzymes responsible for drug and xenobiotic metabolisms. These three families account for about 70% of total CYPs in human livers while CYP4 is a family of enzymes involved in fatty acid and prostaglandin metabolism. Those isoforms include CYP 1A1, 1A2, 2B1, 2B2, 2C11, 2C12, 2E1, 3A1 and 3A2 in rats as well as CYP 1A1, 1A2, 2B6, 2C9, 2D6, 2E1 and 3A4 in humans (Soucek and Gut, 1992; Avenue, 2000). Major CYP enzymes in human, their endogenous substrates, specific substrates, inducers and inhibitors of drug metabolism are shown in Table 3. Examples of rat and human CYPs that activate some potential carcinogens/mutagens are demonstrated in Table 4.

Table 2 The principal reactions in the biotransformation of xenobiotics (Ioannides, 1996)

Reactions	Enzymes	Localization	Substrates
Phase I Reactions: Transformations			
Oxidation	Mixed-function oxidases	Microsomes	Alkanes,alkenes, arenas, amines,thiones, thioethers
	Monoamine oxidases	Mitochondria	Amines
	Alcohol dehydrogenases	Cytosol	Alcohols
	Aldehyde dehydrogenases	Cytosol	Aldehydes
Reduction	Mixed-function oxidases	Microsomes	Azo and nitro groups, N-oxides, arene oxides, alkyl halogenides
	Alcohol dehydrogenases	Cytosol	Aldehydes, ketones
Hydrolysis	Esterases	Cytosol Mitochondria Microsomes	Esters
Phase II Reactions: Conjugations with:			
H_2O	Epoxide hydrolase	Microsomes, Cytosol	Epoxides
Glutathione (GSH)[a]	Glutathione transferases	Microsomes	Electrophiles
Glucuronic acid (UDPGA)[a]	Glucuronyl transferases	Microsomes	Phenols, thiols, amines Carboxylic acids
Sulfuric acid (SAM)[a]	Sulfotransferases	Cytosol	Phenols, thiols, amines
Methyl group (SAM)[a]	N- and O-methyl tranferases	Cytosol, microsomes	Phenols, amines
Acetic acid (Acetyl-CoA)[a]	N-acetyl transferases	Cytosol	Amines

[a] Abbreviations in brackets are co-substrates. UDPGA = uridine-3',5'-diphospho glucuronic acid; SAM = S-adenosylmethionine; CoA = coenzyme A.

CYP 1A subfamily

CYP 1A subfamily consists of two members, CYP 1A1 and CYP 1A2. In general, the CYP 1A subfamily is involved in oxidative metabolism of exogenous substances, such as polycyclic hydrocarbons, heterocyclin amine and aromatic amines (Kawajiri and Hayashi, 1996; Oinonen and Lindros, 1998). CYP 1A1 is expressed not only in the liver, but also in various extrahepatic tissues such as the lung, kidney and placenta, whereas the expression of CYP 1A2 is mainly confined to the liver (Omiecinski, 1999; McLemore, 1990). CYP 1A1 is induced in both liver and extrahepatic tissues by exogenous chemicals, such as 3-methylcholanthrene (3-MC), β-naphthoflavone, 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) and other polycyclic hydrocarbons (Hankinson *et al.*, 1993; Kimura *et al.*, 1986; Nelson *et al.*, 2003; Goshman, 1999; Oinonen and Lindros, 1998). In contrast, CYP 1A2 is primarily expressed in liver at significant constitutive levels and is highly inducible in this tissue *via* the Ah receptor pathway by environmental compounds such as cruciferous vegetables and polycyclic aromatic hydrocarbon (PAHs). Drugs substrates include caffeine, propranolol, theophylline, phenacetin, olanzapine, ondansentron and paracetamol (Kunze and Trager, 1993; Goshman, 1999; Yan and Caldwell, 2001; Parkinson, 2001; Omiecinski *et al*, 1999).

CYP 2B subfamily

CYP2B subfamily comprised ~17 different numbers, identified in several different species. CYP2B1 and CYP2B2 are the primary members expressed in rats, whereas CYP 2B6 is expressed at low levels in human liver. CYP2B1 and CYP2B2 are highly similar in nucleotide sequence and have similar substrate specificity. In rodents, enzymes from this subfamily are typically inducible by phenobarbital and other barbiturates, and are inhibited by metyrapone. Rat CYP2B1 is analogous to human CYP2B6, which generally exists in small amount. CYP2B members are involved in the metabolism of a variety of pharmaceutical compounds, including amphetamines and benzodiazepines. It appears that the ability of phenobarbital to stimulate biotransformation of xenobiotics in human largely stems from its ability to induce other CYPs, CYP2C and CYP3A4 (Parkinson, 2001; Omiecinski *et al.*, 1999).

CYP 2E subfamily

CYP2E1 is the predominant member of the CYP2E subfamily, although CYP2E2 also have been identified in rabbits. CYP2E1 accounts for ~ 6% of the total CYP contents in rat liver, although its expression has been detected in other tissues (Parkinson, 2001). CYP2E1 expression is regulated by several xenobiotics, including ethanol, acetaminophen, carbon tetrachloride, chloroform, isoniazid, halothane, acetone, pyradine and pyrazole (Patten *et al.*, 1989; Koop, 1992: Goshman *et al.*, 1999; Ronis, 1996). CYP 2E1 participates in the metabolism of many endrogenous substances, such as lipid peroxidation products (Terelius and Sundberg, 1986; Oinonen and Lindros, 1998), ketones and polyunsaturated fatty acids, such as linoleic and arachidonic acids (Koop and Cassazza, 1985; Laethem *et al*, 1993). Mechanisms of 2E1 induction include transcriptional activation, mRNA stabilization, translation efficiency, and enzyme stabilization (Berthou, 2001).

CYP3A Subfamily

The CYP 3A subfamily represent about 30% of the total CYP content in human liver (Shimada *et al.*, 1994; Pelkonen and Breimer, 1994; Parkinson, 2001). This subfamily consists of three members (Nelson *et al.*, 1996): CYP 3A4 is the most abundant CYP enzyme in the human liver and it is expressed in several tissues, but the expression in the liver and in the small intestine is of major interest in view of the metabolism of drugs and other xenobiotic chemicals (Guengerich, 1999), CYP 3A5 is a minor polymorphic CYP 3A enzyme, which is expressed in the lungs (Kivisto *et al.*, 1996; Anttila *et al.*, 1997) and the colon (Gervot *et al.*, 1996). CYP 3A7 is expressed in the fetal liver and in the adult endometrium and placenta (Schuetz *et al.*, 1993; Hakkola *et al*, 1994). CYP 3A is inducible by many drugs, for example, rifampicin, dexamethasone, carbamazepine and phenobarbital (Pelkonen *et al.*, 1998; Omiecinski *et al.*, 1999). These enzymes can also be inhibited by a number of compounds including azole antifungals, macrolide antibiotics, gestedene, ethynylestradiol, statins and dihydropyridine (Zuber *et al.*, 2002). This subfamily of enzymes is responsible for metabolisms of a large and diverse group of substrates.

Phase II metabolisms or conjugations generally follow phase I activation, resulting in xenobiotics that have been transferred into water-soluble (hydrophilic) compounds that can be excreted through urine or bile. Several types of conjugation reactions are present in the body, including glucuronidation, sulfation, glutathione and amino acid conjugation (Gibson and Skett, 2000; Liska, 1998). Enzymes of those reactions are located in either cytosol or microsome or both as shown in Table 2.2 (Ioannides, 1996). Glucuronidation is a major pathway of xenobiotic biotransformations in most mammalian species (Parkinson, 2001). In addition, glutathione conjugation has been extensively studied as a major detoxification system and considered as an important protective mechanism against chemical induced carcinogenesis. Toxic electrophiles are mostly deactivated by glutathione conjugation, the reaction which is catalyzed by glutathione-S-transferase, with glutathione acting as a cofactor (Parkinson, 2001; Gibson and Skett, 2000; Ioannides, 1996).

Table 3 Human CYP enzymes, their endogenous substrates, specific substrates, inducers and inhibitor of drug metabolism (Meyer, 1996; Goshman *et al.*, 1999; Avenue, 2000)

Cytochrome P450 isoforms	Drug substrates	Marker substrates/ Reactions	Inhibitors	Inducers
CYP 1A1	R-Warfarin	7-Ethoxyresorufin	7,8-benzoflavone	Omeprazole, Cruciferous vegetables
CYP 1A2	Amitriptyline,betaxolol, caffeine,clomipramine, clozapine,chlorpromazine, fluvoxamine,haloperidol, imipramine,olanzapine, ondansetron,propanolol, tacrine,theophyline, verapamil,(R)-warfarin, acetaminophen,tamoxifen	Phenacetin O-deethylation, 7-Ethoxy resorufin	Enoxacin, ciprofloxacin, grepafloxacin, fluvoxamine, fluoxetine, nefazodone, cimetidine, ofloxacin, ticlopidine	Crigarette smoke, phenobarbital, ritonavir, carbamazepine, charbroiled foods,vegetable, omeprazole
CYP 2A6	Barbiturates, coumarin, nicotine	Coumarin 7-hydroxylation	Ditiocarb sodium (diethyldithiocarbamate)	Barbiturates
CYP 2C9	Diclofenac, flurbiprofen, losartan, phenytoin, piroxicam, tienilic acid, tolbutamide, torasemide, (S)-warfarin, demadex,fluoxetine, ibuprofen,naprofen	Tolbutamide methyl hydroxylation	Amiodarone, fluvastatin, fluvoxamine, fluoxetine, fluconazole, miconazole, metronidazone, ritonavir, sulfamethoxazole	Rifampin, carbamazepine, ethanol, phenytoin, barbiturates
CYP 2C19	Diazepam, (S)-mephenytoin, omeprazole, pentamidine, propranolol, (R)-warfarin	(S)-mephenytoin 4-hydroxylation		Carbamazepine, norethindrone

Table 3 (con't) Human CYP enzymes, their endogenous substrates, specific substrates, inducers and inhibitor of drug metabolism (Meyer, 1996; Goshman *et al.*, 1999; Avenue, 2000)

Cytochrome P450 isoforms	Drug substrates	Marker substrates/ Reactions	Inhibitors	Inducers
CYP 2D6	Bufuralol, codeine, debrisoquine, desipramine, dextromethorphan, encainide, fluoxetine, haloperidol, imipramine, nortriptyline, paroxetine, propafenone, propranolol, sparteine	Bufuralol 1-hydroxylation	Quinidine, ajmaline, amiodarone, fluoxetine, amiodarone, haloperidol, ritonavir	Rifampin
CYP 2E1	Paracetamol, caffeine, chlorzoxazone, enflurane, theophylline	Chlorzoxazone 6-hydroxylation	Dithiocarb sodium	Alcohol (ethanol), isoniazid
CYP 3A	Benzphetamine, clarithromycin, codeine, cyclosporin, dapsone, diazepam, erythromycin, felodipine, tacrolimus, indinavir, lovastatin, midazolam, nifedipine, carbamazepine, losartan, quinidine, taxol, terfenadine, verapamil	Testosterone 6β-hydroxylation	Gestodene, troleandomycin, ketoconazole, itraconazole erythromycin, clarithromycin, cimetidine, grapefruit juice	Barbiturates, rifampicin, dexamethasone, carbamazepine, phenytoin,

Table 4 Carcinogens and toxins bioactivated by human P450s (Gonzalez and Gelboin, 1994)

Cytochrome P450 isofroms	Carcinogens/toxins
CYP 1A1	Benzo(a)pyrene, dimethylbenz(a)anthracene, 6-nitrochrysene
CYP 1A2	2-Aminofluorene, 2-acetylaminofluorene, aflatoxin B_1, 4-aminobiphenyl, 2-aminoanthracene, 2-amino-3-methylimidazo[4,5 *f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5 *f*]quinoxaline ($MeIQ_x$), 2-amino-3,4,8-trimethylimidazo[4,5 *f*]quinoxaline ($DiMeIQ_x$), 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-pyridol[4,3-b]indole (Trp P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp P-2), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK), 6-nitrochrysene
CYP 2A6	Aflatoxin B_1, N-nitrosodiethylamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK)
CYP 2B6	6-Aminochrysene
CYP 2C8, 9, 18	None known
CYP 2D6	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK)
CYP 2E1	Acetaminophen, acrylonitrile, benzene, carbon tetrachlorine, chloroform, 1,2-dichloropropane, ethyl carbanate, ethylene dibromine, ethylene dichloride, methyl chloride, methylene chloride, N-nitrosodiethylamine, styrene, vinyl carbamate, vinyl bromide, vinyl chloride, 1,11-trichloroethane, trichlorethylene
CYP 3A4	Aflatoxin B_1, aflatoxin G_1, 6-aminochrysene, benzo(a)pyrene-7,8 diol, 6-nitrochrysene, 1-nitropyrene, tris-(2,3-dibromopropyl)phosphate, sterigmatocystin, serecionine

Mechanism of induction of CYP

Induction is defined as an increase in amount and catalytic activity of CYP. Classically, induction is an adaptive response that protect cells from toxic xenobiotics by increasing the detoxification activity. While in the most cases, CYP induction is the consequence of an increase in gene transcription, some non-transcriptional mechanisms are also known to be involved. For example, troleandomycin induces human CYP3A4, but the mechanism is not transcriptional. Troleandomycin produces no increase in the rate of CYP3A4 protein synthesis, but it decreases the rate of CYP3A4 protein degradation. Similarly, induction of CYP2E1 by alcohol (ethanol), acetone and isoniazid is caused by a non-transcriptional mechanism. Spontaneously induced diabetic rats and rats with chemically induced diabetes exhibit increased levels of CYP2E1 that appear to reflect mRNA stabilisation and not gene transcription. With the exception of the CYP1A1 isoform, the molecular mechanisms involved in CYP induction are not well understood. In the case of CYP1A1, inducing agents bind to cytosolic polycyclic aromatic hydrocarbon (Ah) receptors and are translocated into the nucleus. The transcriptional process includes a sequence of events: ligand-dependent heterodimerisation between the Ah receptor and an Ah receptor nuclear translocator, interaction of the heterodimer with a xenobiotic-responsive enhancer, transmission of the induction signal from the enhancer to a CYP1A1 promoter, and alteration in chromatin structure. This is followed by subsequent transcription of the appropriate mRNA and translation of the corresponding proteins. In drug therapy, there are 2 major concerns related to CYP induction. First, induction will result a reduction of pharmacological effects caused by increased drug metabolism. Secondly, induction may create an undesirable imbalance between 'toxification' and 'detoxification'. Like a double-edged sword, induction of drug metabolising enzymes may lead to a decrease in toxicity through acceleration of detoxification, or to an increase in toxicity caused by increased formation of reactive metabolites.

Mechanism of inhibition

Many therapeutic drugs and environmental xenobiotics have been reported to inhibit CYP in the liver via different mechanisms. The inhibition effects can take place in

several ways including the destruction of pre-existing enzymes, an inhibition of enzyme synthesis, an inactivating of the drug-metabolizing enzymes and a competitive for the enzyme catalytic sites. The inhibition of drug metabolism may result in undesirable elevations in plasma drug concentrations. Thus, the inhibition of CYP is of clinical importance for both therapeutic and toxicological reasons.

Mechanism of CYP inhibition can be divided into three categories (Lin and Lu, 1998; Williams, 1995):

1. Reversible inhibition

Reversible inhibition is probably the most common mechanism responsible for the documented drug interactions. Generally, this interaction is the result of competition between inhibitor and substrate at the same CYP active site. The effect of this inhibition will be dissipated after discontinuing the inhibitor. Many of the potent reversible CYP inhibitors nitrogen-containing drugs, including imidazoles, pyridines and quinolines. These compounds not only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of inhibitor is determined both by its lipophilicity by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron.

2. Quasi-irreversible inhibition via metabolic intermediate complexation

Quasi-irreversible inhibition occurs when a reactive metabolite forms stable complex with prosthetic heme of CYP. The stable complex is called metabolic intermediate (MI) complex. The MI complex can be reversed and the catalytic activity of CYP can be restored by incubating *in vitro* with lipophilic compounds that can displace the inhibitor from the active site. However, synthesis of *de novo* enzyme is required to restore CYP activity in an *in vivo*. A large number of drugs, including

methylenedioxybenzenes, alkylamines, macrolide antibiotics and hydrazines, undergo metabolic activation by CYP enzymes to form inhibitory metabolites.

3. Irreversible inhibition

Drugs containing certain functional groups can be oxidised by CYP to reactive intermediates that cause irreversible inactivation of the enzyme prior to its release from the active site. Because metabolic activation is required for enzyme inactivation, these drugs are classified as mechanism-based inactivators or suicide substrates. The mechanism-based inactivation of CYP may result from irreversible alteration of haem or protein, or a combination of both. In general, modification of the haem group invariably inactivates the CYP, whereas protein alteration will result in loss of catalytic activity only if essential amino acids, which are vital for substrate binding, electron transfer and oxygen activation, are modified.