CHAPTER II

LITERATURE REVIEWS

Murdannia loriformis

M. loriformis (Hassk.) Rolla Rao et Kammathy, known in Thai as "Ya Pak King" or "Angel Grass", belongs to family Commelinaceae. *M. loriformis* comes from south Chinese and grows in sandy soil with the sunshine outline. In China, plants in the genus *Murdannia* are used to treat bronchitis and relieve ailment of neoplasms. In Thailand, *M. loriformis* is used as a traditional medicine to relief pain from cancer (Salalamp *et al.*, 1992). Examples of effects of *M. loriformis* used in patients with cancer as conventional medicine was shown in Table 1. About 100-120 g of fresh aerial parts of *M. loriformis* are ground with 4 tablespoons of water and the obtained juice is filtered. Patients are suggested to take the filtrate orally twice a day, before breakfast and evening meal (วีณา จิรัจฉริยากูล, 2542).

Morphological characteristic of *M. loriformis*

M. loriformis is a plant of about 7-10 cm high. Its leaves are simple alternate leaves at lower stem with about 1.5 cm wide, 10 cm long, whereas leaves at the upper stem are shorter. Inflorescence is terminal, each floret consists of sepals of 4 mm long, obovate; stamens 2 perfect and 3 staminodes, ovary of 1 mm long, style of 3 mm long. Fruits are capsules of 3-4 mm long, obovoid, with 2 seeds in each cell (Salalamp *et al.*, 1992; วีณา จิรัจฉริยากูล และคณะ, 2536) (Figure 1).

Table 1 Examples of effects of *M. loriformis* used in patients with cancer as conventional medicine (วีณา จิรัจฉริยากูลและคณะ, 2543).

Sex	Age	Year	Disease and symptoms	After used *M. loriformis*
male	64	1984	Spleen and liver cancer, weight loss, anorexia, insomnia	Appetite increment, weight gain, hypnotic
female	58	1986	Cancer of cervix, swelling and pain of legs	Relief of pain, normal movement
male	63	1986	Cancer, hemoptysis	Relief of cough
male	84	1986	Cancer of leg and feet, wound inflammation with pus and lymph discharge	Dryness of wound, without pus and lymph
male	50	1986	Cancer of right leg, wound and pus discharge from toe with bad smell, and leg pain	Appetite increment, weight gain, hypnotic
male	64	1987	Cancer of intestine and liver, hemoptysis, feeling of weakness after operation	Able to get up and walk, appetite increment
male	-	1987	Cancer of liver and cholecyst, jaundice, anorexia, back and waist pain, weight loss, insomnia	Pain relief, appetite increment, hypnotic
female	71	1987	Leukemia, anorexia, weight loss, pale	Appetite increment, weight gain, hypnotic
female	78	1988	Cancer of cervix, lymph discharge from cervix with bad smell, no restrain the urine	Reduce of lymph discharge and bad smell, normal restrain of urine
female	46	1988	Haemorrhoid, intestine contraction, sometine no break wind, pain when defecate	Pain relief when defecate, can break wind, normal defecate

Table 1 *(continued)* Examples of effects of *M. loriformis* used in patients with cancer as conventional medicine (วีณา จิรัจฉริยากูลและคณะ, 2543).

Sex	Age	Year	Disease and symptoms	After used *M. loriformis*
female	39	1988	Cancer of intestine, vomit, anorexia, can not lie on one's back	Relief of vomiting, appetite increment, can lie on one's back
male	19	1988	Leukemia, weakness, weight loss	Increasing of leukocyte, weight gain
male	34	1993	Cancer of liver, pain of right rib, can not sleep on one's side, anorexia	Appetite increment, no growth of cancer cell

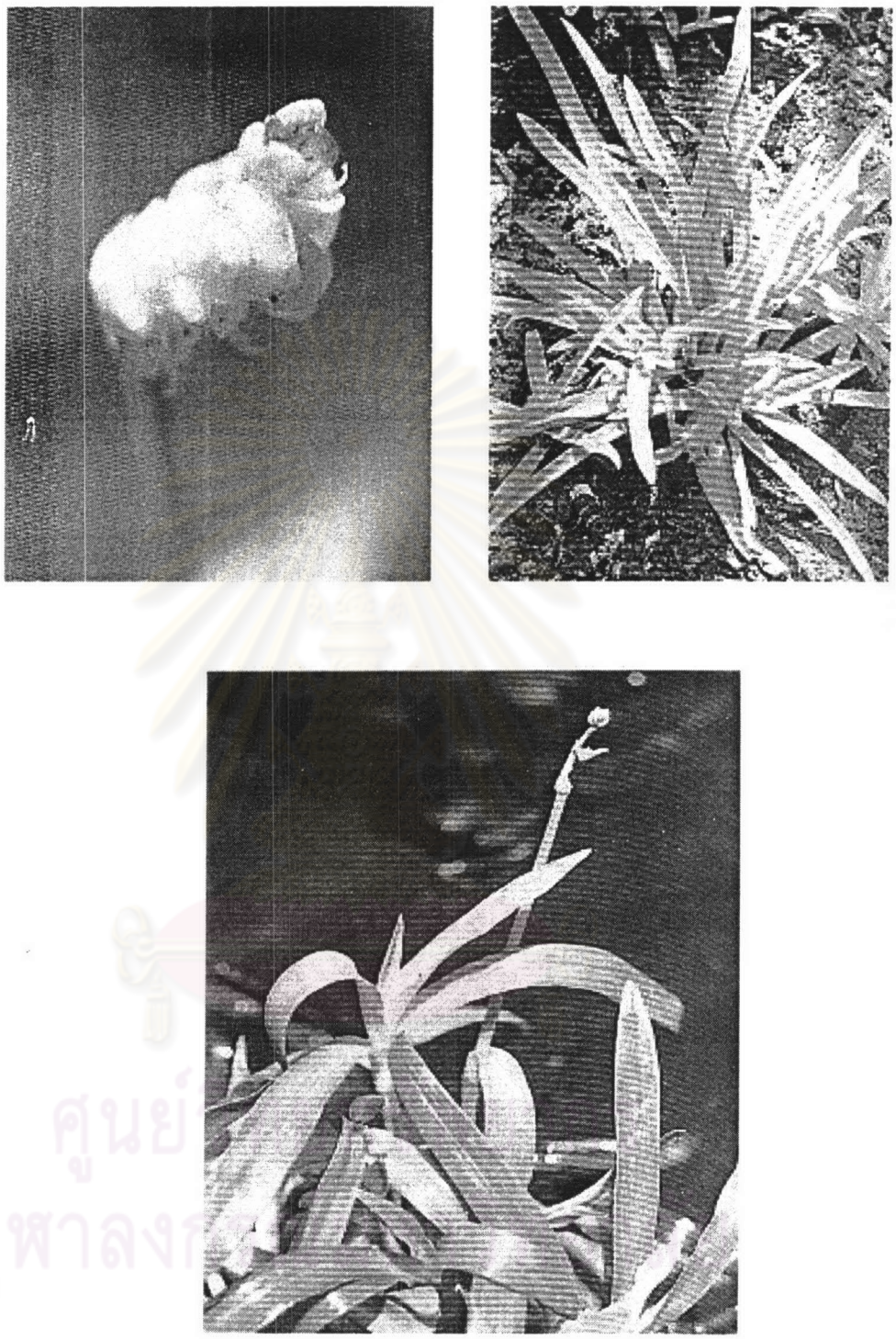


Figure 1 Ya Pak King (*M. loriformis* (Hassk.) Rolla Rao et Kammathy)
(Salalamp *et al.*, 1992)

Chemical constituents found in *M. loriformis* include (วีณา จิรัจฉริยากูล, 2536; Jiratchariyakul, 1997; Narintorn, 1999)

1. **Amino acid**: L-phenylalanine

L-phenylalanine is a protein amino acid. It is classified as an essential amino acid. Dietary sources of the amino acid are principally derived from animal and vegetable proteins. Vegetables and juices contain small amounts of the free amino acids. The free amino acid is also found in fermented foods such as yogurt. L-phenylalanine is the precursor of L-tyrosine. The conversion of L-phenylalanine to L-tyrosine is via the enzyme L-phenylalanine hydroxylase. L-tyrosine can be converted by neurons in the brain to dopamine and norepinephrine (noradrenaline), neurotransmitters which are depleted by stress, overwork and certain drugs. The mechanism of L-phenylalanine is putative antidepressant activity may be accounted for by its precursor role in the synthesis of the neurotransmitters norepinephrine and dopamine. Elevated brain norepinephrine and dopamine levels are thought to be associated with antidepressant effects (Birkmayer *et al.*, 1984).

2. **Phenolic compounds**: syringic acid

Phenolic compounds are universal components of plant material. The main phenolic acids found in foods are derivatives of 4-hydroxybenzoic acid and 4-hydroxycinnamic acid. Syringic acid as derivative of 4-hydroxybenzoic acid. It has been found in grape, plum, and vegetables (Rice-Evans, 2003). Previous study demonstrated that syringic acid exerted a direct antiproliferative action on T47D human breast cancer cells (Kampa *et al.*, 2004).

3. **Flavonoids**: isovitexin, chalconoids

Flavonoids are widely distributed in various species of plants. Numerous groups have investigated the antioxidant properties of flavonoids. Many are also potent inhibitors of several enzymes, such as xanthine oxidase, cyclooxygenase, and lipoxygenase. A major property of flavonoids is their ability to scavenge hydroxyl radicals. Lin *et al.*, (2002) found that isovitexin demonstrated a characteristic of low cytotoxicity (LD_{50} >400 μM), but significant xanthine oxidase inhibitory activity and antioxidative activity, thus exhibited potent protection of cells from xanthine oxidase

induced damage. Chalconoids either natural or synthetic are known to exhibit various biological activities. They have been reported as antibacterial, antifungal, antiinflammatory, antitumor, antimutagenic. Chalconoids serve as precursors for the synthesis of different classes of flavonoids (Mukherjee *et al.*, 2001; Satyanarayana *et al.*, 2004).

4. **Phytosterols**: β-sitosterol, sitosteryl glucoside (3-β-D-glucopyranosyl-24ξ-ethyl-cholest-5-ene)

Plant kingdom contains a number of sterols that differ from cholesterol by giving ethyl or methyl groups or unsaturation in the side chain. Three phytosterols including sitosterol, stigmasterol and campesterol are ubiquitous in higher plants. They are all essential components of plant membranes. Beta-sitosterol is the major phytosterol in higher plants, and is found in the serum and tissues of healthy individuals at concentrations 800-1000 times lower than that of endogenous cholesterol. Its glycoside, β-sitosterol glycoside, is also present in serum in even lower concentrations. These molecules are synthesized in plants whereas animals obtain them through diet.

The scientific literature is replete with reports of the biological activities of sterols or their glycosides in various animal models. For instance, β-sitosterol and its glycoside have been shown to reduce carcinogen-induced cancer of the colon in rats, as well as exhibit various effects of anti-inflammatory, anti-pyretic, insulin releasing, and immune modulation (Bouic and Lamprecht, 1999).

5. **Glycosphingolipids**: 1-β-O-D-glucopyranosyl-2-(2′-hydroxy-6′-cosamide)-sphingosine

Glycosphingolipids are classified as polar lipids. They are the important part of biological membrane. They differ from other lipids in that they are water soluble. They occur mainly in brain, nervous system and other organs such as red blood cells, kidneys, spleen, placenta, serum and liver. Generally glycosphingolipids play a role in immune reaction. Glycosphingolipids isolated from the cancer cell are different from those isolated from the normal cell. The difference indicates the altered immune reaction of cancer cell. They are thus the most interesting in part of cancer research (Jiratchariyakul *et al.*, 1998). Glycosphingolipids, which are major membrane constituents, play important roles in cell recognition, cell adhesion, and as receptors of

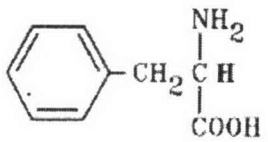
microorganisms. These compounds change during cell differentiation and oncogenic transformation, and some are recognized as differentiation or tumor markers (Narintorn, 1999).

6. Digalactosyl diglyceride

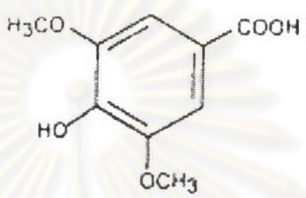
Digalactosyl diglyceride have galactose as the sugar group and are also known as galactolipids, which is classified in glycolipid. The glycolipids are a structurally heterogeneous group of membrane components found in all species. The term "glycolipid" designates any compound containing one or more monosaccharide residues bound by a glycosidic linkage to a lipid moiety which as hydrophobic moiety such as an acylglycerol, a sphingoid, a ceramide (*N*-acylsphingoid) or a prenyl phosphate. The galactolipids, mono- and digalactosyl diglyceride, are the major constituents of thylakoid membranes accounting for 80% of polar lipids found in this membrane. It either is synthesized directly in the plastid or is assembled from diacylglycerol moieties imported from the endoplasmic reticulum and is the main glycolipid components of the various membranes of chloroplasts and related organelles, and indeed these are the most abundant lipids in all photosynthetic tissues, including those of higher plants, algae and certain bacteria (Christie, 2002).

Chemical structures of these constituents in *M. loriformis* were shown in Figure 2.

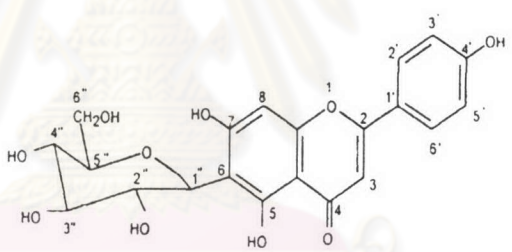
Amino acid: L-phenylalanine



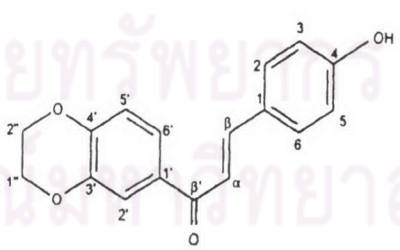
Phenolic compound: syringic acid



Flavonoid: isovitexin, chalconoid



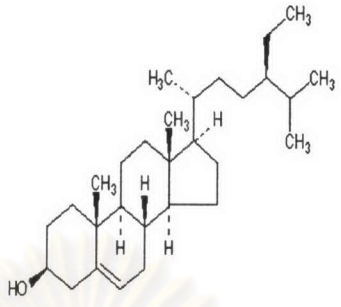
Apigenin-6-C-β-D-glucopyranoside (isovitexin)



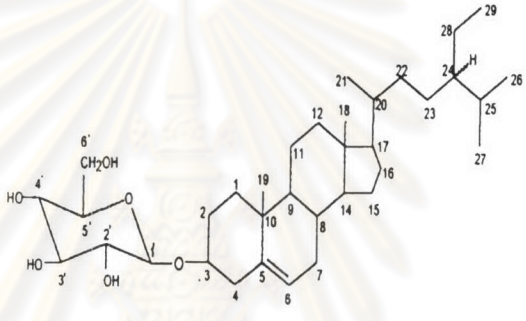
Chalconoid

Figure 2 Chemical constituents found in *M. loriformis* (Jiratchariyakul, 1997; Narintorn, 1999).

Phytosterols: β-sitosterol, sitosteryl glucoside (3-β-D-glucopyranosyl-24ξ-ethyl-cholest-5-ene)



β-sitosterol



3-β-D-glucopyranosyl-24ξ-ethyl-cholest-5-ene

Glycosphingolipids: 1-β-O-D-glucopyranosyl-2-(2′-hydroxy-6′-cosamide)-sphingosine

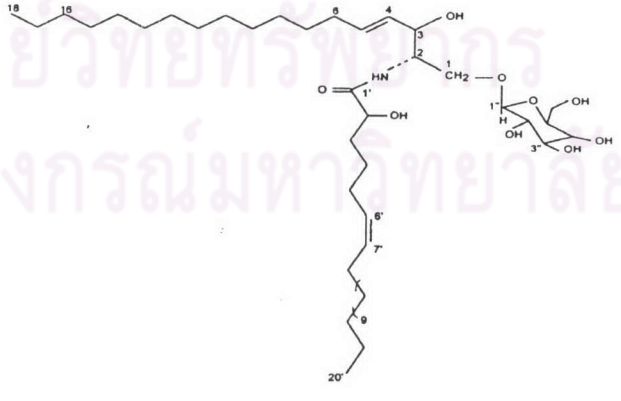


Figure 2 *(continued)* Chemical constituents found in *M. loriformis* (Jiratchariyakul, 1997; Narintorn, 1999).

Digalactosyl diglyceride

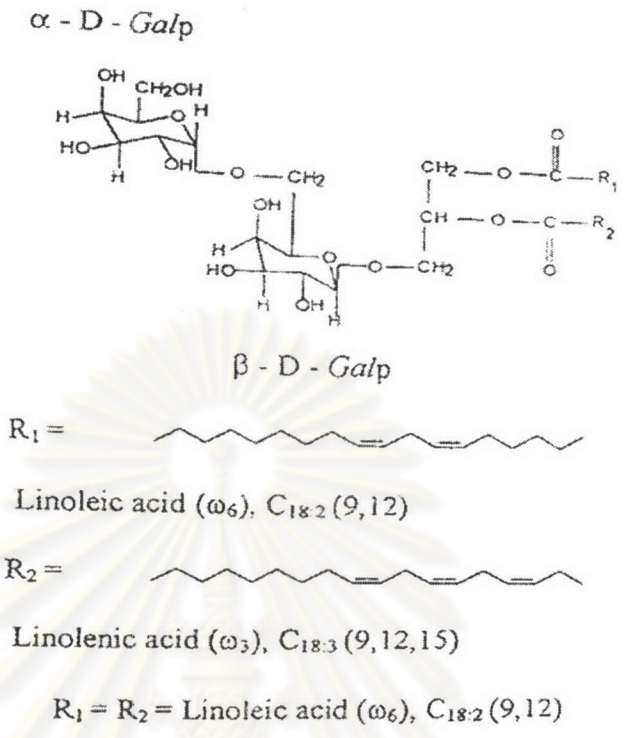


Figure 2 *(continued)* Chemical constituents found in *M. loriformis* (Jiratchariyakul, 1997; Narintorn, 1999).

Toxicological effects

Acute toxicity

An acute toxicity study was performed in rats using pressed juice from fresh *M. loriformis* at doses of 5, 10, 20 and 30 g/250 g body weight. The results showed that no abnormal signs were observed during 24 hours through 14 days after treatment. Clinical blood chemistry and histopathological findings of lungs, kidneys, spleens, livers and testis/ovaries were also within normal limits. Median lethal dose (LD_{50}) was found to be greater than 120 g/kg of body weight which was equivalent to 300 times of therapeutic dose used in man (พิมลวรรณ ทัพยุทธพิจารณ์ และคณะ, 2533).

Subchronic toxicity

A subchronic toxicity study was performed in rats using pressed juice from fresh *M. loriformis* at doses of 0.7, 1.75, 3.5 g/250 g body weight for 3 months. The results showed that no abnormal signs were observed. Clinical blood chemistry and histopathological findings of lungs, kidneys, spleens, livers and testis/ovaries were also within normal limit (พิมลวรรณ ทัพยุทธพิจารณ์ และคณะ, 2534).

Pharmacological effects

1. Cytotoxic effect

Dried powder of *M. loriformis* was extracted with petroleum ether, then extracted with chloroform and ethanol, respectively. The ethanol extract was fractionated by Diaion HP20 (MCI gel CHP20P) column and eluted into water, water-methanol (1:1), methanol and ethyl acetate fractions. The water fraction contained inorganic salts, carbohydrates and amino acids. The water-methanol (1:1) fraction contained phenolic compounds. The methanol fraction contained glycosidic compounds, named phytosterol glucoside (G1a), glycosphingolipid (G1b), named 1-β-O-D-glucopyranosyl-2-(2′-hydroxy-6′-cosamide)-sphingosine) and digalactosyl diglyceride (G2a and G2b). The MTT cytotoxicity test was performed on these fractions. Results showed that phytosterol glucoside and digalactosyl diglyceride were not cytotoxic while glycosphingolipid exerted cytotoxicity (ED_{50} less than 10 μg/ml) against human colon carcinoma (SW 620) and human breast cancer (BT 474) cell lines (Jiratchariyakul *et al.*, 1997; Jiratchariyakul *et al.*, 1998).

2. Antimutagenic effect

In an *in vitro* study, ethanolic extract of *M. loriformis* had no mutagenic effects on *Salmonella typhimurium* TA100 and TA98 either with or without metabolic activation. The extract inhibited mutagenesis induced by alfatoxin B_1 (AFB_1), 2-amino-6-methyldipyrido[1,2-a:3',2'-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) as determined by Ames' test. In contrast, the extract did not inhibit mutagenesis induced by sodium azide (NaN_3), 2-(2-furyl)-3-(5-nitro-2-furyl) furamide (AF-2) and benzo(a)pyrene (B(a)P). Also, the ethanolic extract of *M. loriformis* induced DT-diaphorase activity in murine hepatoma cell lines (Vinitketkumnuen *et al.*, 1996). DT-diaphorase has been known to be able to detoxify a number of natural and synthetic compounds, including quinones and their derivatives, as well as to protect cells against oxidative stress (Lind *et al.*, 1990). Current evidence suggests that induction of chemoprotective enzymes such as DT-diaphorase by any

compounds is the predominant mechanism by which these compounds are able to be used as a chemoprotective agent (Riley *et al.*, 1992).

Likewise, 80% ethanolic extract of *M. loriformis* showed antimutagenicity against 2-amono-3-methylimidazo[4,5-*f*]quinoline, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole, 2-amino-6-methyldipyrido[1,2-a:3',2'-*d*]imidazole, 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole, 2-aminoanthracene, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, *N*-methyl-*N*'-nitrosoguanidine and methylazoxymethanol acetate. The inhibitions of mutagenicities were ranged between 31.4-67.9% at the dose of 10 mg/plate. However, the extract did not inhibit the mutagenesis induced by 2-amino-9*H*-pyridol[2,3-*b*]indole, 2-amino –3-methyl-9*H*-pyridol[2,3-*b*]indole, benzo(a)pyrene, *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine and 1-nitropyrene (Intiyot *et al.*, 2002).

3. Chemopreventive effect

Chemopreventive effect of 80% ethanolic extract of *M. loriformis* was performed at doses between 0.1-1.0 g/kg on aberrant crypt focus formations in male rat colon which were induced by subcutaneously injection of 15 mg/kg body weight of azoxymethane, a principally colon carcinogen in rodents. The results showed that the extract at doses between 0.1-1.0 g/kg significantly inhibited azoxymethane induced aberrant crypt focus formation both in the initiation stage (21-51%) and post-initiation stage (12-27%) in rat colon (Intiyot *et al.*, 2002). Aberrant crypt focus is postulated to be the earliest identifiable potential precursor of colon cancer. Analysis of aberrant crypts may facilitate the study of the early pathological and molecular changes that precede colon cancer (Yamada *et al.*, 2001; Pereira *et al.*, 1994).

4. Hypoglycemic effect

Hypoglycemic effect of *M. loriformis* was investigated *in vivo* using water extract, hot water extract and ethanolic extract of this plant in glucose-induced hyperglycemic mice compared to a standard oral hypoglycemic drug, glibenclamide. Blood glucose monitoring was measured every 30 minutes for 3 hours using blood sampling from mice's tail. The results showed that hot water extract and ethanolic extract of *M.*

loriformis given orally at 2 g/kg body weight significantly reduced blood glucose of mice. Hot water extract showed the maximum effect at 30 minutes after administration and the effect lasted for 60 minutes. Ethanolic extract produced maximum effect at 60 minutes which was later than that of the hot water extract. Ninety minutes after administration, the hypoglycemic activity of this plant extracts disappeared (เอื้อพร ไชยวรรณและคณะ, 2544).

5. Immunomodutator effect

A study was performed to investigate the effect of pressed juice from *M. loriformis* on human immune-mediated cytotoxicity. The results showed that the pressed juice had no cytotoxic effect on the viability of peripheral blood mononuclear and increased proliferation of lymphocyte. It increased the cluster of differentiation (CD) on the surface of the T-lymphocyte (CD3,4 and CD3,8) after incubation with peripheral blood mononuclear at 37°C, 5% CO_2 humidify for 3 days. However, the cluster of differentiation (CD) on the surface of the T-lymphocyte (CD3,4 and CD3,8) was decreased if the incubation prolonged to 7 days. Explanation for the inconsistency of the results was still inconclusive (วีณา จิรัจฉริยากูลและคณะ, 2543).

Xenobiotics metabolism

Xenobiotics are foreign chemical compounds to the body, such as drugs, food additives, and environmental pollutants (Murray, 1993). Most of these compounds are subjected to be metabolized in the body. Xenobiotic metabolism is a biological process which alters many substances leading to termination or alteration of biological activity. Xenobiotic-metabolizing enzymes occur in many organs. Liver is the main organ involved in metabolism. Other organs that display this function include gastrointestinal tract, lungs, skin, and kidneys. Enzymes are found in smooth endoplasmic reticulum (SER). Some are located in cytosol and a few are found in other organelles such as mitochondria. Xenobiotic biotransformation enzymes convert chemicals of lipophilic property to metabolites of hydrophillic property that are more readily excreted in urine or feces (Timbrell, 2000; Parkinson, 2001). In general, the reactions catalyzed by these enzymes are divided into phase I and phase II reactions and sometimes, phase III reactions (Table 2).

Table 2 The major biotransformation reactions (Timbrell, ed., 2000)

Phase I	Phase II	Phase III
Oxidation	Sulphation	Further metabolism of
Reduction	Glucuronidation	Glutathione conjugates
Hydrolysis	Glutathione conjugation	
Hydration	Acetylation	
Dehalogenation	Amino acid conjugation	
	Methylation	

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.

Phase I reactions

Phase I reactions introduce a functional group (-OH, -NH_2, -SH or -COOH, etc.) to a molecule leading to a small increase in hydrophilicity as well as a suitable characteristic for further phase II biotransformations (Timbrell, 2000; Parkinson, 2001). Phase I reactions change many xenobiotics to more polar metabolites which are more active, less active or inactive than the parent compounds. The reactions involved in this process include oxidation, reduction, and hydrolysis. Xenobiotic oxidation is mediated mainly by CYP, flavin-containing monooxygenase, peroxidases, prostaglandin synthetase, lipoxygenases, and processes occurring during lipid peroxidation-coupled co-oxidation. A few additional pathways (e.g., dehydrogenase, amine oxidase) of xenobiotic oxidation are known. NADPH cytochrome P-450 reductase, azoreductase, nitroreductase, and other reductases bring about reduction of xenobiotics, while epoxide hydrase, esterases, and amidases effect hydrolytic cleavage of specific linkages in the xenobiotic molecule (Hood, 1997). The reactions which mostly catalyzed by CYP monooxygenase enzymes. These enzyme systems are predominantly localized in the membrane of SER of the liver cells, thus known as microsomal enzymes. These enzymes generally catalyze the oxidation reactions of a wide variety of both endogenous compounds and xenobiotics with overlapping substrate specificity (Potter and Coon, 1991; Guengerich, 1991, 1992). Major CYP enzymes in human, their specific substrates and their percent participation in drug metabolism are shown in Table 3.

Cytochrome P-450

The CYP comprise a superfamily of hemoproteins. They share an identical prosthetic group (a thiolate-bound heme) and mechanism of catalysis (activation of oxygen without activating the substrate), but differ widely in apoprotein structure, which endows unique substrate specificities. Amino acid sequences of genes are used to classify CYP into families and subfamilies. The CYP enzymes are membrane-bound haem proteins, which catalyse mono-oxygenation reactions. They are bound either to the microsomal membrane or, in some cases, to the mitochondrial inner membrane. Mitochondrial CYPs, involved in steroid-biosynthetic reactions, are found mainly in steroidogenic organs and generally do not metabolize foreign compounds. The majority

of microsomal CYPs belonging to families 1, 2 and 3 metabolize a great variety of structurally different xenobiotics such as drugs, alcohols, aromatic organic compounds, including many environmental pollutants and natural plant products. Some of these are chemical carçinogens or mutagens. 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 prostaglandins metabolism (Rendic and Di Carlo, 1997). CYP isoforms which play a role in the activation of xenobiotics to toxic metabolites include CYPs 1A1, 1A2, 2B1, 2B2, 2E1, 3A in rats as well as CYPs 1A1, 1A2, 2B6, 2E1, 3A4 in humans. An example of rat and human CYPs that activate some potential carcinogens/mutagens are demonstrated in Table 4.

The expression of CYPs is highest in the liver, both by quantity and by CYP diversity, but they are also expressed at a lower level in kidney, lung, intestine, brain, placenta, etc. In the liver the CYP enzymes are constitutively expressed and commonly also induced by chemicals.

The basic reaction catalyzed by CYP is a monooxygenation reaction as following (Gibson and Skett, 2000):

$$\text{Substrate (RH)} + O_2 + \text{NADPH} + H^+ \xrightarrow{\text{CYP}} \text{Product (ROH)} + H_2O + NADP^+$$

Product from this reaction does not appear to be only a simple alcohol because rearrangement might be occurred. There are also documented that CYP may catalyze reduction reaction e.g. carbon tetrachloride, azo dyes and epoxides (Guengerich, 1991). However, biotransformation by CYP is not always a detoxification reaction. A variety of specific CYP isoforms, especially CYP in family 1, 2 and 3 are involved in the activation of certain chemical procarcinogens (Soucek and Gut, 1992; Parkinson, 2001).

The regulation of these enzymes has been most extensively studied using rat, mouse and rabbit as model systems. Species differences have been noted in the expression of CYP gene. Table 5 presents sequential homology between rat and human CYP isoforms known at the end of 1990. Sequential homology of cDNA and amino acid sequence between rat and human CYP isoforms is high in similarity, approximately 70%. There are generally conserved regions (for P-450 reductase, haem, signal peptide)

which increase this similarity. On the other hand, change in a single amino acid may markedly or even completely alter CYP function, such as increase or decrease its activity, or even completely change its substrate specificity (Soucek and Gut, 1992).

Important members of CYP families

CYP1A Subfamily

Enzymes in CYP1A subfamily are responsible for metabolic activation of some known procarcinogenic environmental chemicals, toxins, and drugs. CYP 1A1 and 1A2 are the most important members of this family. *CYP 1A1* gene is not normally expressed in liver, but it dramatically (up to 1000-fold) induced by polycyclic aromatic hydrocarbons (PAHs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), benzo(a)pyrene, methylcholanthrene (MC), β-naphthoflavone (β-NF), that are also metabolized by this CYP isoform. CYP1A1 is expressed and induced in several extrahepatic tissues, including lungs, and its capacity to activate tobacco-smoke-derived PAHs is the basis for the putative role of this CYP isoform in lung carcinogenesis (Oinomen and Lindros, 1998). In contrast to CYP 1A1, CYP1A2 is not expressed in extrahepatic tissues. *CYP1A2* gene is constitutively expressed in liver and is also induced by aromatic hydrocarbons. It activates arylamines and dietary promutagens, including nitrosamines and aflatoxins. CYP1A1 and CYP1A2 are found in both humans and rats. Function of CYP1A is fairly well conserved across species, although there are subtle differences (Parkinson, 2001). For instance, isolated and purified human CYP1A2 enzyme from the liver has been shown to display substrate specificity similar to the rat protein. The human CYP1A2 isoform is functionally important, being involved in the metabolism of several clinically important drugs such as acetaminophen, amitriptyline, propanolol, theophylline, warfarin, etc. Some drugs are inducers (e.g. omeprazole and lansoprazole), or inhibitors (e.g. amiodarone, cimetidine, ticlopidine) of this CYP isoform (Parkinson, 2001).

CYP2B Subfamily

The expression of the highly similar CYP2B1 and 2B2 isoforms is low in rat liver, but both are up to 50-fold induced by phenobarbital (Oinomen and Lindros, 1998). CYP2B1 and CYP2B2 are highly similar in nucleotide sequence (with less than 3% sequence differences) and have similar substrate specificity (Soucek and Gut, 1992). Rat CYP2B1 is analogous to human CYP2B6 that is found in liver and extrahepatic tissues but are generally expressed at low levels in the absence of inducers such as phenobarbital. CYP 2B6 is found to metabolically activate 6-aminochrysene, 3-methoxy-4-aminoazobenzene and antineoplastic drugs (e.g. cyclophosphamide, ifosfamide) (Gonzalez and Gelboin, 1994). Pentoxyresorufin and benzyloxyresorufin are often used as substrate probes to measure CYP2B activity *in vitro* (Omiecinski *et al.*,1999). Inhibitors of CYP 2B6 are 9-ethynylphenathrene, methoxyclor and orphenadrine (Parkinson, 2001).

CYP2E Subfamily

CYP 2E1 is expressed constitutively in liver and possibly in extrahepatic tissues, such as kidney, lung, lymphocytes and bone marrow (Parkinson, 2001). This enzyme is capable of metabolizing numerous low molecular weight chemicals, including benzene, carbon tetrachloride, acrylonitrite, N-nitrosodimethylamine, and others. The ubiquitous nature of CYP 2E1 substrates in the industrial workplace, environment, tobacco smoke, and diet has marked human health implication (Gonzalez and Gelboin, 1994). Only a few substrates are drugs such as acetaminophen, dapsone, theophylline, chlorzoxazone (Parkinson, 2001). CYP2E1 substrates such as ethanol, isopropanol, acetone, toluene, and benzene, may also induce CYP2E1 itself. Isoniazid and imidazole compounds are also potent inducers. Diabetes induce the activity of this enzyme (Rendic and Di Carlo, 1997). In human, CYP2E1 is accounted for approximately 6% of total hepatic CYP. This isoform can vary up to 20-fold among individuals, probably due to induction by environmental factors. Mechanisms of CYP2E1 induction include transcriptional activation, mRNA stabilisation, translation efficiency, and enzyme stabilisation (Berthou, 2001). Human liver CYP2E1 is similar to rat CYP2E1 and rabbit

CYP2E1 in structure, catalytic activity and regulatory characteristics (Wrighton *et al.*, 1986). Thus, CYP2E1 are well conserved among mammalian species (Parkinson, 2001).

CYP3A Subfamily

The most abundant P450 enzymes in human liver microsomes belong to the *CYP 3A* gene subfamily, which include CYP3A4, CYP3A5, and CYP3A7. CYP3A4 and CYP3A5 are expressed in liver and in intestinal mucosa (Omiecinski *et al.*, 1999). CYP3A7 is represented in fetal liver, adult endometrium, and placenta. CYP3A subfamily members are accounted for 30% of total CYP content in human livers (Taavitsainen, 2001). CYP3A are very important in metabolism of drugs and are involved in metabolic activation of a number of chemical carcinogens including aflatoxin B_1, benzo(a)pyrene 7,8-diol, 1-nitropyrene, 6-aminochrysene, and the chemotherapeutic agent such as cyclophospharnide, ifosfamide. CYP3A4 is the major P450 expressed in human and figures prominently in the metabolism of drugs. More than 150 drugs belonging to about 38 classes are listed as substrates of CYP3A4. Examples of substrates are opioid analgesics, corticosteroids, immunosuppressants and antiarrhythmics. This enzyme also catalyzes the metabolism of endogenous steroids including androgens, anabolic hormones, cortisol, estradiol and progesterone (Table 3).

In human, CYP 3A enzymes are inducible by numerous drugs, such as rifampin, phenobarbital, phenytoin and troglitazone. Inhibitors of human CYP3A include azole type antimycotics (e.g., ketoconazole and clotrimazole), macrolide antibiotics (e.g., erythromycin), HIV protease inhibitors (especially ritonavir), gestodene, ethynylestradiol, statins, and dihydropyridine calcium channel blockers, grapefruit juice (containing the flavonoids quercetin, naringin, bergamottin and naringenin) (Parkinson, 2001; Zuber *et al.*, 2002).

CYP3A1 and CYP3A2 are found in rats. Expression of CYP3A1 is very low in normal rat liver, whereas CYP 3A2 is the adult male-specific CYP which is absent from the livers of adult females (Oinomen and Lindros, 1998).

Table 3 Human CYP enzymes, their specific substrates and their percent participation in drug metabolism (modified from Rendic and Di Carlo, 1997)

CYP enzymes	Substrates	Participation in drug metabolism (%)
1A1	7-Methoxyresorufin	2.5
	R-Warfarin	
1A2	Acetaminophen	8.2
	Caffeine	
	7-Ethoxyresorufin	
	7-Methoxyresorufin	
	Phenacetin	
	R-Warfarin	
2B6	Cyclophosphamide	3.4
	7-Benzyloxyresorufin	
	S-Mephenytoin	
	7-Pentoxyresorufin	
	Testosterone	
2E1	Acetaminophen	4.1
	Aniline	
	Chlorzoxazone	
	Dapsone	
	Halothane	
	p-Nitrophenol	
2C8, 9	Diclofenac	15.8
	Hexobarbital	
	Phenytoin	
	Tolbutamide	

Table 3 *(continued)* Human CYP enzymes, their specific substrates and their percent participation in drug metabolism (modified from Rendic and Di Carlo, 1997)

CYP enzymes	Substrates	Participation in drug metabolism (%)
2C8, 9	S-Warfarin	
2C18, 19	Diazepam	8.3
	S-Mephenytoin	
	Omeprazole	
2D6	Codeine	18.8
	Bufuralol	
	Debrisoquine	
	Dextromethophane	
	Sparteine	
3A4, 5	Carbamazepine	34.1
	Cortisol	
	Dapsone	
	Diazepam	
	Erythromycin	
	Midazolam	
	Nifedipine	
	Omeprazole	
	Testosterone	

Table 4 Role of rat and human CYPs in the activation of some potential carcinogens/ mutagens (Soucek and Gut, 1992; Guengerich, 1993; Gonzalez and Gelboin, 1994)

CYP	Potential mutagens/ carcinogens	
	Rat	Human
1A1	Aflatoxin B_1 Benzo(a)pyrene 7,12-Dimethylbenz(a)anthracene 2-Naphthylamine 4,4'-(bis) Methylene chloroaniline	Benzo(a)pyrene 7,12-Dimethylbenz(a)anthracene 6-Nitrochrysene
1A2		2-Acetylfluorene 2-Aminoanthracene Aflatoxin B_1 4-Aminobiphenyl 2-Naphthylamine 6-Nitrochrysene
2B1	2-Acetylfluorene Aflatoxin B_1 Benzo(a)pyrene 3-Methylcholanthrene 4,4'-(bis) Methylene chloroaniline	
2B2	4,4'-(bis) Methylene chloroaniline	
2B6		6-Aminochrysene
2B7		Aflatoxin B_1
2E1	N-N'-Nitrosodimethylamine N-Nitroso-N-diethylamine	Acrylonitrile Benzene Carbon tetrachloride Chloroform

Table 4 *(continued)* Role of rat and human CYPs in the activation of some potential carcinogens/ mutagens (Soucek and Gut, 1992; Guengerich, 1993; Gonzalez and Gelboin, 1994)

CYP	Potential mutagens/ carcinogens	
	Rat	Human
2E1		N-Nitroso-N-diethylamine
		Styrene
		Trichloroethylene
		Vinyl carbamate
		Vinyl bromide
		Vinyl chloride
3A4		Aflatoxin B_1
		Aflatoxin G_1
		Benzo(a)pyrene
		6-Nitrochrysene
		Sterigmatocystin

Table 5 Sequential homology between rat and human CYP forms (Soucek and Gut, 1992)

P-450 form		Sequential homology[a]
Rat	Human orthologue	(%)
CYP 1A1	CYP 1A1	80(78)
CYP 1A2	CYP1A2	75(70)
CYP 2A1	_____	—[b]
CYP2A2	_____	—[b]
CYP2A3	CYP2A6	(85)
_____	CYP2A7	—[b]
CYP2B1	CYP2B6	78(74)
	CYP2B7	(76)
CYP2B2	_____	—[b]
CYP2B3	_____	—[b]
CYP2C6	CYP2C10	(75)
CYP2C11	CYP2C9	80(77)
CYP2C12	_____	—[b]
CYP2C13	CYP2C8	74(68)
CYP2D1	CYP2D6	(71)
CYP2E1	CYP2E1	75(78)
CYP3A1	CYP3A3	(78)
_____	CYP3A4	(73)
CYP3A2	CYP3A5	(71)
	CYP3A7	(65)
CYP3A9	_____	—[b]
CYP4A1	CYP4A9	—[b]
CYP4A3	_____	—[b]
CYP11A1	CYP11A1	79(76)

[a]Similarity of cDNA and amino acid (in parentheses) sequence stated.

[b]No data available regarding existence of orthologous form.

Mechanism of induction of CYPs

Induction is defined as an increase in amount and catalytic activity of CYP. Classically, definition of induction is a *de novo* synthesis of new enzyme molecules as a result of an increase transcription of its gene after stimulation by an appropriate chemical signal. An increase in enzyme activity due to activation is not usually included under the term of induction. Enyzme induction is dose-dependent, generally with a steep dose-response relation and no clear-cut threshold of no-effect (Woolf, 1999).

A considerable diversity has been depicted in the mechanisms of regulation of CYP (Figure 3). Mechanisms of induction include gene transcription through receptors, mRNA processing, mRNA stabilisation and enzyme stabilisation which are shown in Table 6 (Berthou, 2001).

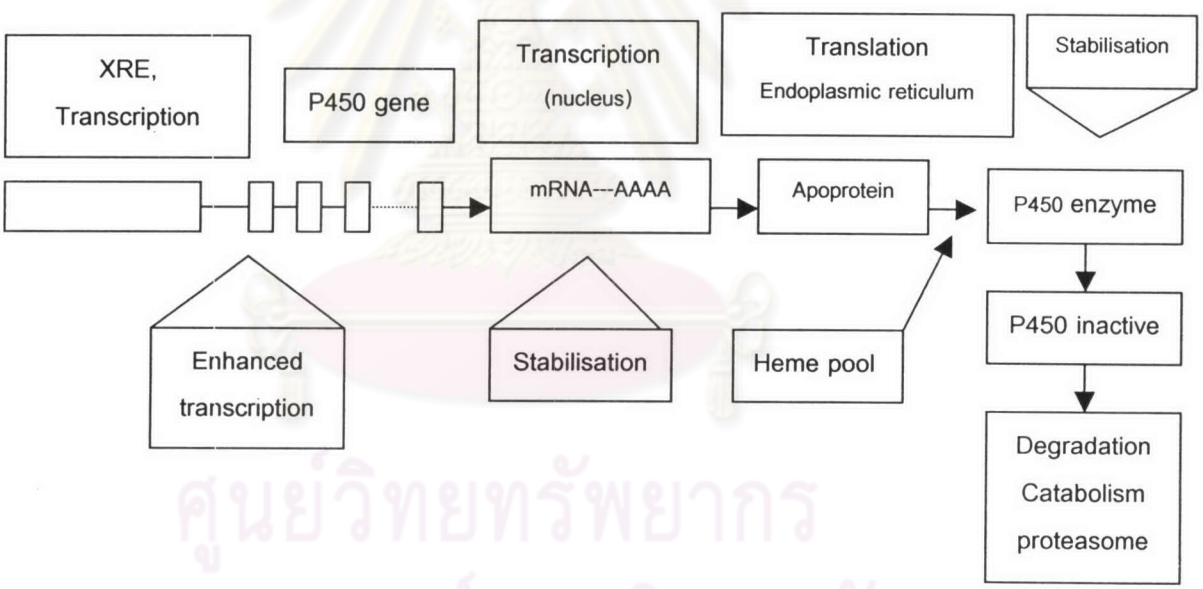


Figure 3 Steps in P450 gene expression (Berthou, 2001)

Effect of induction can be seen within the first two days of compound exposure, but it usually takes more than a week for new enzymes to be synthesized and the maximal effect to occur. The time course of enzyme induction onset and offset is closely related to the plasma concentration of the inducer, as well as the half-life of enzyme production and degradation. For drugs that are active in their parent forms, enzyme induction can enhance metabolic rate of the affected drug, resulting in a decrease in the

serum concentration of parent drug, and possibly a loss of clinical efficacy. For prodrugs, compounds that require metabolic activation and whose effects are produced by the metabolites, enhanced pharmacodynamic effects may be expected (Berthou, 2001).

Table 6 Mechanisms of induction known to date for different CYPs (Berthou, 2001)

Induction mechanism	CYPs known to be induced
Gene transcription through receptors	1A1 (cytosolic AhR), 1A2, 1B1 2A6, 2B6 (CAR), 2C8, 2C9, 2C18, 2C19 3A4, 3A5 (nuclear receptors PXR and SXR) 4A11 (PPAR ∝)
mRNA processing	1A2
mRNA stabilisation	1A1, 2E1, 3A4
Enzyme stabilisation	2E1

AhR = aryl hydrocarbon receptor

CAR = constitutive androstane receptor

PPAR = peroxisome proliferator activated receptor

PXR = pregnane (or prenenolone)-X-receptor

SXR = steroid xenobiotic receptor

Mechanism of inhibition of CYPs

(Berthou, 2001; Lin and Lu, 2001; Gibson and Skett, 2000; Woolf, 1999)

Enzyme inhibition results in a decrease in the clearance, thereby an increase in the steady-state serum concentration of the affected drug. The magnitude of the effect is largely unpredictable because it depends on the specific enzyme which is inhibited and the quantitative importance of that pathway in the overall clearance of the affected drug. Inhibition of CYP enzymes is the most common cause of metabolism based drug-drug interactions. The inhibition of CYP enzymes is of clinical importance for both therapeutic and toxicological reasons. The mechanisms of CYP inhibition can be categorized into reversible inhibition, quasi-irreversible inhibition, mechanism-base inhibition .

1. Reversible inhibition

Reversible inhibition is the most common type of enzyme inhibition. Reversible inhibition is transient and reversible, and the normal functions of CYPs continue after the inhibitor has been eliminated from the body. Reversible inhibition can be further classified into competitive, uncompetitive, mixed-type and non-competitive inhibition. Competitive inhibition is when the binding of an inhibitor to an enzyme prevents a further binding of a substrate to the active sites of the enzyme. In uncompetitive inhibition, an inhibitor does not bind to the free enzyme, but binds to the enzyme-substrate complex, resulting in a nonproductive enzyme-substrate-inhibitor complex. Mixed-type inhibition is when an inhibitor binds either to the free enzyme or to the enzyme-substrate complex. In the case of noncompetitive inhibition, an inhibitor binds to a nonactive binding site of the enzyme, and the binding has no effect on the binding of substrate, but the enzyme-substrate-inhibitor complex is nonproductive.

2. Quasi-irreversible inhibition *via* metabolic intermediate complexation

Quasi-irreversible inhibition occurs when a reactive metabolite forms a 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*.

3. Mechanism-base inhibition

Mechanism-based inhibition can be mediated by covalent modification of a pyrrole nitrogen in the prosthetic heme group of CYP or by direct modification of the heme moiety or the apoprotein. The mode of inhibition is highly specific because the inhibitor must both bind to and be metabolised by the enzyme. The inhibitory effect of mechanism-based inhibition is terminated by enzyme resynthesis rather than inhibitor washout.

One mode of mechanism-based inhibition is the so-called enzyme inactivation (or suicide inhibition). Suicide inhibition results from covalent binding of reactive intermediates to the heme and/or protein of CYP.

The most important phenomena of mechanism-based inhibition are time, concentration, and NADPH-dependent loss of the enzyme activity. *In vivo*, the inhibitory effect of a mechanistic inactivator is thought to be more prominent after repeated dosing and last longer than that of a reversible inhibitor.