

CHAPTER II

LITERATURE REVIEWS

2.1 Overview of caffeic acid and its derivatives

Caffeic acid (3, 4-dihydrocinnamic acid, CAF) is one of the most common phenolic acid which is found in fruits, grains, dietary supplements and Chinese medicinal herbs. The structure of CAF is shown in Fig. 2.1. CAF derivatives including amides, esters, sugar esters and glycosides are widely distributed in coffee beans, olives, propolis, fruits and vegetables [14]. The physiological function of CAF and its derivatives have been studied by many researchers which are summarized herein:

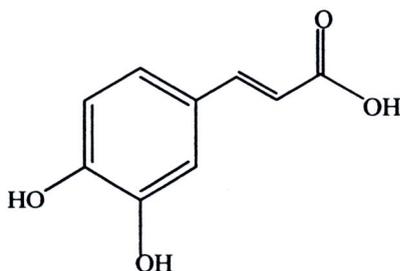


Fig. 2.1 The structure of caffeic acid.

2.1.1 Antioxidant effects

CAF is a strong scavenger against superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced by various chemicals. Cheng *et al.* [15] studied hydrocinnamic acid such as CAF, chlorogenic acid (ChA), sinapic acid (SA), ferrulic acid (FA) and p-coumaric acid (CoA) on peroxidation of human low density lipoprotein induced by 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) or cupric ion. Kinetic analysis of the antioxidation process demonstrated that hydrocinnamic acid are effective antioxidant against both AAPH and cupric ion-induced LDL peroxidation with the activity sequence of CAF~ChA>SA>FA>CoA and CAF>ChA>SA>FA~CoA, respectively.

Many researchers reported the effect of CAF and its derivatives on the radical scavenging activity. Wu *et al* [16] and Gulcin [17] extensively investigated the antioxidant properties of CAF and/or its derivatives and found that they could scavenge DPPH radical, superoxide anion and ABTS radical *in vitro* models. Moreover, CAF was highly effective as an antioxidant substance by total reductive capability using the potassium ferric cyanide reduction method, and by total antioxidant activity using ferric thiocyanate method.

Son and Lewis [14] synthesized CAF amide and ester analogues and investigated antioxidants and free radical scavenging properties. The results showed that DPPH radical scavenging activity of the test compounds were as followed: N-trans-caffeoyl-L- cysteine methyl ester > N-trans-caffeoyldopamine > N- trans-caffeoyltyramine> N-trans-caffeoyl- β -phenethylamine > trolox > caffeic acid phenethyl ester (CAPE) > CAF > ferrulic acid. The result established that the radical

scavenging activities of the compounds were increased as a result from the numbers of hydroxyl groups. In term of antioxidative activity by using emulsified linoleic acid oxidation system accelerated by AAPH, CAPE exhibited the highest potency among synthetic substances. Wang *et al.* [18] showed CAPE and its derivatives were screened for their ability to scavenge intracellular reactive oxygen species generated in HUVEC cells by measuring 5-(and-6)-chloromethyl-2', 7'-dichlorodihydro fluorescein diacetate oxidation. ROS levels were reduced in the cells when compared with the control.

2.1.2 Inhibition of mutagenicity

Propolis is a broad spectrum of activities such as antibiotic, antiviral and anti-tumor. CAF is one component presented in propolis. Rao *et al.* [19] synthesized three CAF ester derivatives; methyl caffeate (MC), phenethyl caffeate (PEC) and phenethyl dimethyl caffeate (PEDMC) and tested against 3, 2'-dimethyl-4-aminobiphenyl (DMAB)- induced mutagenicity in *Salmonella typhimurium* strain TA98 and TA 100. The results indicated that MC, PEC and PEDMC were not mutagenic in the Salmonella tester system. DMAB-induced mutagenicity was significantly inhibited with 150 μ M MC, 40–60 μ M PEC and 40–80 μ M PEDMC in both tester systems. Treatment of HT-29 colon adenocarcinoma cells with concentration higher than 150 μ M MC, 30 μ M PEC and 20 μ M PEDMC significantly inhibited the cell growth and synthesis of RNA and DNA and activity of protein ornithine decarboxylase and protein tyrosine kinase.

2.1.3 Modulation of metabolizing/detoxifying enzymes

Most procarcinogens require metabolic activation by metabolic enzymes including phase I and phase II enzymes in order to convert to electrophile before they can exert carcinogenic effects [20]. Wang *et al.* determined the activity of CAPE and six catechol ring-fluorinated CAPE derivatives on cytoprotective ability and heme oxygenase -1 (HO-1) activity using HUVEC cells line. Modification of the catechol ring of CAPE by insertion of fluorine at various positions resulted in dramatic changes in cytoprotective activity. CYP 1A1/2 and CYP2B1/2 activity in male Fischer-344 rats treated with CAPE were decreased [21].

2.1.4 Inhibition of activity related to tumor promotion, proliferation and mitotic signal transduction

Lee *et al.* [22] synthesized 14 derivatives of phenethyl ester and tested for anti-cancer activity by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric and tryphan blue dye exclusion assay on the growth of oral squamous cell carcinoma (SAS), oral epidermoid carcinoma-Meng 1 (OEC-M1) and normal human oral fibroblast (NHOF cells). CAPE and analogues showed cytotoxic effects on SAS and OEC-M1 cell lines, but no effect to NHOF cell lines at 5 to 100 μM . Flow cytometric analysis showed that CAPE analogues caused OEC-M1 cell arrest at G2/M phase and might be useful in oral cancer chemotherapy.

2.2. Overview of free radicals and antioxidants

A free radical is a molecule or ion containing one or more unpaired electrons. Examples of free radicals are shown in Table 2.1. All living cells are exposed constantly to oxidants from both endogenous and exogenous source and free radicals are continuously produced *in vivo* [23]. Oxidation and production of free radicals are an integral part of human metabolism. Reactive oxygen and nitrogen species can attack with biomolecules in the cells including cell membrane, lipid, nucleic acid and intracellular proteins.

Table 2.1 Examples of free radicals found in biological system

Name	Formula	Comments
Hydroxyl radical	OH^\bullet	A highly reactive oxygen-centered radical which attack all molecules in human body.
Nitrogen oxide radical	$\text{NO}^\bullet, \text{NO}_2^\bullet$	Nitric oxide is formed by L-arginine <i>in vivo</i> model. Nitrogen dioxide is produced when NO reacts with O_2 and is found in air pollution and burning organic materials including cigarette.
Peroxyl, alkoxy radicals	$\text{RO}_2^\bullet, \text{RO}^\bullet$	Oxygen-centered radicals formed during the breakdown of organic peroxides.
Superoxide radical	O_2^\bullet	An oxygen –centered radical, highly reactive

2.2.1 Oxidative stress and antioxidants

Oxidative stress can be started as imbalance between pro-oxidant/ free radical production and opposing antioxidant defenses. Example of acute and chronic oxidative stress are atherosclerosis, diabetes mellitus, Alzheimer's disease, inflammatory disease, carcinogenesis, pulmonary disease and hematological diseases [24,25]. ROS can attack on cell membrane, intracellular proteins, enzymes, carbohydrate, and DNA as shown in Fig 2.1. In biological system, an antioxidant has been defined as any significant substance that delay or prevents oxidation reaction. Antioxidant may intervene at any of the three major steps of the free radical mediated oxidative process including initiation, propagation and termination. Antioxidants can classify as antioxidant enzyme, endogenous non-enzymatic antioxidants and exogenous antioxidant molecules [26].

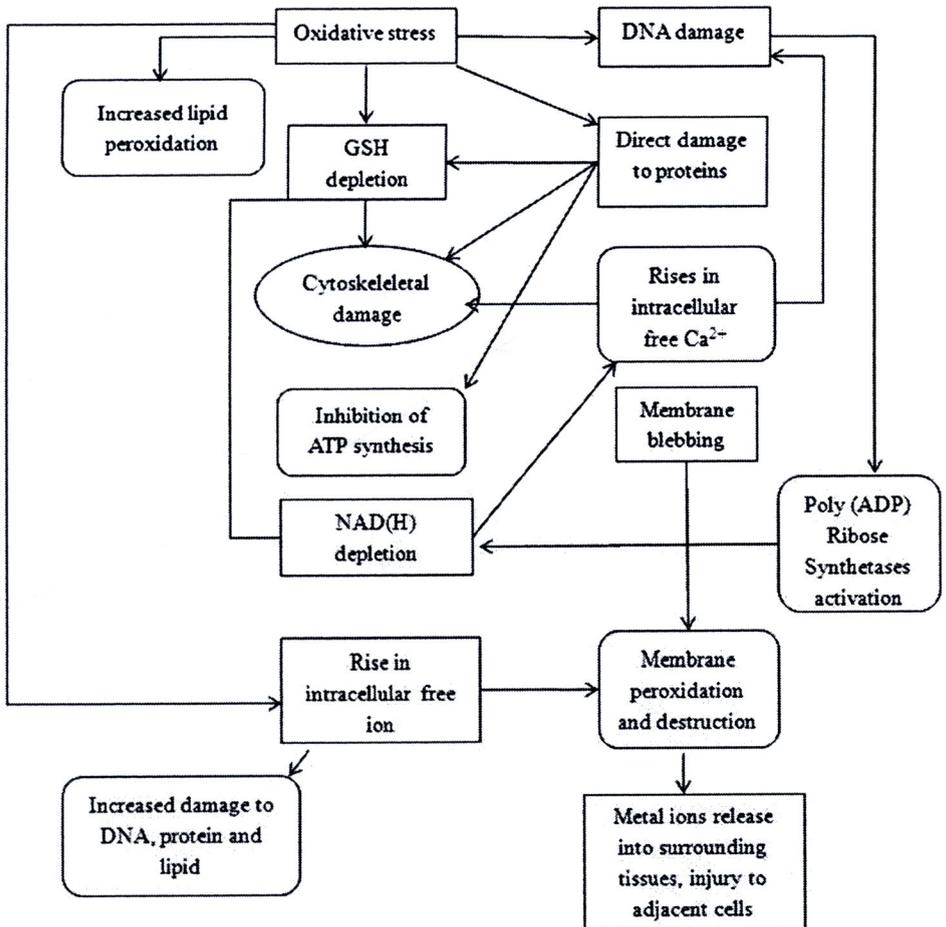


Fig. 2.2 The interacting mechanism of cell injury by oxidative stress. [26]

2.2.3 Antioxidant defense systems

2.2.3.1 Antioxidant enzymes

The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are the main of cellular antioxidant defence system. SOD changes superoxide radical to hydrogen peroxide. CAT detoxifies hydrogen

peroxide to water and oxygen. GPX reduced both hydrogen peroxide and organic hydroperoxides by using reduced glutathione (GSH) as the electron donor. In the absence of oxidative stress, cellular free radicals are kept at low level through the balanced, co-ordinated action of these three enzymes. Both the decrease in antioxidant enzyme activity and unbalanced overexpression of one of these enzymes such as SOD, can increase the vulnerability of mammalian cells to free radicals [27].

2.2.3.2 Endogenous non-enzymatic antioxidants

Non-enzymatic antioxidants provide the primary defence against extracellular and intracellular free radicals. They include a variety of lipophilic (e.g. bilirubin) and hydrophilic (e.g. GSH, uric acid) molecules or protein components (sulphydryl groups on cysteine) that act as free radical scavengers. GSH is a tripeptide which acts on multiple levels of the antioxidant defence:

1. As a scavenger of free radicals such as superoxide radical, hydroxyl radical and lipid hydroperoxides
2. As a substrate for the antioxidant enzyme
3. In the direct repair of antioxidative DNA lesions [27].

2.3 Antioxidant activity assay

It is necessary to determine the efficacy of natural or synthetic antioxidants for preservation and protection against oxidative damage. It is desirable to develop a rapid method for determining the potential antioxidant capacity in foods. The methodologies used for evaluating natural antioxidants must be carefully interpreted

based on the system and analytical method for determining the extent of end point of oxidation. Due to difficult in measuring each antioxidant component separately, and interaction among these different antioxidant components in the network, several methods have been developed to assess the total antioxidant capacity of all the nonenzymatic antioxidant components in biological samples. The effectiveness of an antioxidant is measured and the extents of antioxidation by chemical, instrumental or sensory methods were estimated [27].

2.3.1 *In vitro* methods for antioxidant assay

2.3.1.1 *DPPH* assay

2, 2-diphenyl-1- picrylhydrazyl (DPPH) is a stable nitrogen free radical and this assay based on measurement of antioxidant scavenging capacity. The nitrogen electron on DPPH molecule is reduced by donating hydrogen atom from antioxidants to hydrazine. The ability is evaluated using electron spin resonance spectroscopy on basis that the DPPH radical signal intensity is inversely proportional to the antioxidant concentration and time. However, the discoloration assay, which evaluates decrease in absorbance at 515 to 528 nm produced by adding hydrogen atom to DPPH radical is more frequently used method.

The DPPH method is a valid, easy, accurate, sensitive and inexpensive method to evaluate antioxidant scavenging activity of fruits, vegetables and herbal extracts. The results are highly reproducible and comparable to other scavenging methods such as ABTS. However, DPPH is also sensitive to some Lewis base and solvents. Oxygen may react with DPPH directly in presence of light and decrease its

absorbance. DPPH is soluble only in organic solvents and interference of absorbance from sample compounds could be a problem for quantitative analysis. The method has a limitation in reflecting the partitioning of antioxidant, in emulsion systems and is not useful for measuring the antioxidant activity of plasma as protein are precipitated in alcoholic medium[26,28].

2.3.1.2 Ferric reducing antioxidant power assay

Ferric reducing antioxidant power or ferric reducing ability of plasma (FRAP) method is used for assessing antioxidant power of antioxidants. The FRAP assay directly measures the ability of antioxidant to reduce ferric tripyridyltriazine complex (Fe^{3+} -TPTZ) to ferrous complex (Fe^{2+} -TPTZ) at low pH. In this method, excess Fe^{3+} is used and the rate-limiting factor of Fe^{2+} -TPTZ and color formation is the reducing ability of the samples. The FRAP assay is simple, precise, sensitive, low cost and reproducible results with both individual and mixture antioxidants. However, the measured reducing capacity dose not reflects to antioxidant activity. Since, the method dose not includes any oxidizable substrate, no information is provided on the protective properties of the antioxidants [28].

2.3.1.3 Inhibition of induced lipid autoxidation

This method artificially induces linoleic acid or low density lipoprotein (LDL) autoxidation by Cu (II) or an azo compound initiator. The progress of autoxidation is monitored by UV absorbance at 234 nm which is maximum



absorbance of conjugated diene peroxides from linoleic acid oxidation. The reaction can be carried out in micelles or in organic solvents. In micelles, reaction progress cannot be followed directly by a UV spectrometer and sample workup is necessary that is the efficiency limit of this method. Typically, the assay solution contains free radical initiator, 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH), linoleic acid as substrate, antioxidant and ambient oxygen [28]

2.3.2 Free radical scavenging methods

2.3.2.1 Superoxide anion radical scavenging assay

Superoxide anion is a weak oxidant which can generate both hydroxyl radicals and singlet oxygen. Numerous biological reactions generate superoxide anions which are highly toxic species. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. Gallic acid, BHA, ascorbic acid, α -tocopherol, curcumin, quercetin and trolox can be used as a positive control [29].

2.3.2.2 Hydrogen peroxide radical scavenging assay

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as

a bleaching agent in the textile, paper and pulp industries. Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals that can initiate lipid peroxidation and cause DNA damage. The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch *et al.* [30]. Ascorbic acid, rutin BHA, α -tocopherol and quercetin can be used as a positive control.

2.3.2.3 Nitric oxide radical scavenging assay

In vitro model, nitric oxide generated from sodium nitroprusside in aqueous solution pH 7.4 with oxygen to produce nitrite ions. Nitric oxide was measured using the Griess reaction reagent composing of (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) [31]. Curcumin, CAF, BHT and α -tocopherol can be used as a positive control.

2.3.2.4 Hydroxyl radical scavenging assay

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. The model used in generating $HO\cdot$ is ascorbic acid-iron- EDTA system. This system composed of ascorbic acid, iron and EDTA. Gallic acid,

mannitol, catechin, vitamin E, quercetin, BHA, α -tocopherol, rutin and ascorbic acid can be used as a positive control [32].

2.4 Overview of xenobiotic metabolism

Chemicals biotransformation is an important process consisting of both bioactivation and detoxification [33]. There are two general types of reactions that xenobiotics compounds are denoted as phase I and phase II metabolism. Phase I metabolism is the enzymatic transformation by oxidation, hydroxylation, dealkylation and reduction reaction to add functional group on foreign compounds. The cytochrome P450 is the most important enzyme in phase I metabolism which has many different isoforms and metabolizes various chemicals. Phase II metabolism transforms the structure of xenobiotics or phase I metabolite via conjugation with endogenous substances including glucuronic acid, glutathione, amino acid and sulfate.

2.4.1 Phase I enzyme: Cytochrome P450s

The cytochrome P450s (CYP) enzyme consists of a superfamily of haemoprotein. CYPs play role in the oxidative transformation of endogenous and exogenous compounds [34]. In all CYP, the prosthetic group is constituted of Fe (III) protoporphyrin IX covalently linked to the protein by the sulfur atom of a proximal cysteine ligand as shown in Fig 2.3.

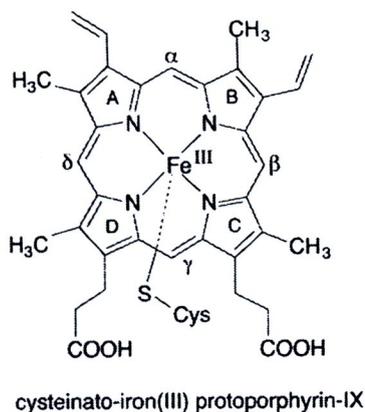


Fig. 2.3 Prosthetic of haemoprotein: Fe (III) protoporphyrin-IX linked with a proximal cysteine ligand [34]

The CYP enzyme family plays an important role in phase I xenobiotic metabolism. The CYP superfamily consisted of more than 7,000 named sequence in animal, plants, bacteria and fungi [35]. The human genome has 57 CYP genes, but 15 individual CYP enzymes in family 1, 2 and 3 that metabolize xenobiotics [36]. There are evidences shown that expression patterns of many individual CYPs in different tissues and cell types of organs have important role in physiology [37]. CYP enzymes are found in practically all tissues, with the highest abundance and the largest number of CYP isoforms present in the liver. Furthermore, CYPs reside in the intestine, lung, kidney, brain, adrenal gland, gonads, heart, nasal and tracheal mucosa and skin [38]. In human liver, CYP enzymes composed approximately 2% of total microsomal protein. Each CYP isoform is a specific gene product with different substrate specificity. In human liver, there is at least 12 isoforms CYPs and only six isoenzymes from the families CYP1, 2 and 3 which are involved in the hepatic metabolism of most of the drugs such as CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6 and CYP2E1[39,40].

2.4.1.1 Cytochrome P450 1A2 (CYP1A2)

CYP1A2 accounts for approximately 15% of total CYP protein content in human liver [41]. CYP1A2 metabolizes more than 20 clinically used drugs including theophylline, clozapine, propranolol, phenacetin and acetaminophen. Human CYP1A2 has been purified from liver microsomes as an enzyme catalyzing phenacetin *O*-deethylation [42]. An extensive investigation of various procarcinogen, it was concluded that the enzyme mainly activated aromatic amines including, 2-acetylaminofluorene (2-AAF), 2-aminoanthracene (2-AA), 2-aminofluorene (2-AF) and 2-naphthylamine (2-NA) and heterocyclic amine, for example, 2-amino-6-methylimidazo [1, 2-a:3'2'-d] imidazole (Glu-P-1), 2-aminodipyrido [1,2-a: 3'2'-d] imidazole (Glu-P-2) and 2-amino-3-methylimidazo [4,5-f] quinoline [IQ] [43]. Moreover, CYP1A2 appears to be a major human hepatic enzyme involved in the activation of cigarette smoke condensate, and aflatoxin B1 (AFB1), a potent hepatocarcinogen and ubiquitous dietary contaminant in some countries.

2.4.1.2 Cytochrome P450 2E1 (CYP2E1)

CYP2E1 is involved in metabolism of low molecular weight toxins, fluorinated ether volatile anesthetics and procarcinogens. This isozyme is inducible by ethanol and responsible in part of acetaminophen metabolism which the metabolite is highly reactive and hepatotoxic. In addition, CYP2E1 has unique capacity to reduce dioxygen to reactive oxy radicals that might initiate lipid oxidation. The highest CYP2E1 concentrations are found in the endoplasmic reticulum. The CYP2E1 expression is highest in the liver, where most of the protein is found in the

perivenous region, in the four to five layers of hepatocytes surrounding the hepatic vein. CYP2E1 is also present in the kidney, nasal mucosa, lung, testis, ovary, brain, small intestine, colon, endothelial cells and lymphocytes [44, 45].

2.4.1.3 Cytochrome P450 3A4 (CYP3A4)

The CYP3A4 enzyme, which represents the majority of CYP protein in the human liver, is responsible for the metabolism of many endogenous including testosterone, progesterone, cortisol, oestradiol and exogenous compounds (steroids, antidepressants, antibiotics, benzodiazepines, calcium channel blocker, HMG-Co A and anticancer drugs). Four human CYP3A genes have been identified: CYP3A4, CYP3A5, CYP3A7 and CYP3A43 which cluster on chromosome 7. Based on the amount of protein and catalytic potential, CYP3A4 is the most important member of CYP3A subfamily. Furthermore, CYP3A4 metabolizes procarcinogen and other toxins such as aflatoxin B1, aflatoxin G1, 6-nitrochrysene, 1-nitropyrene and sterigmatocystin [43, 46, 47].

Cytochrome P450 and reactive oxygen species

CYPs can generate (ROS) by the catalytic cycle as shown in Fig 2.4.

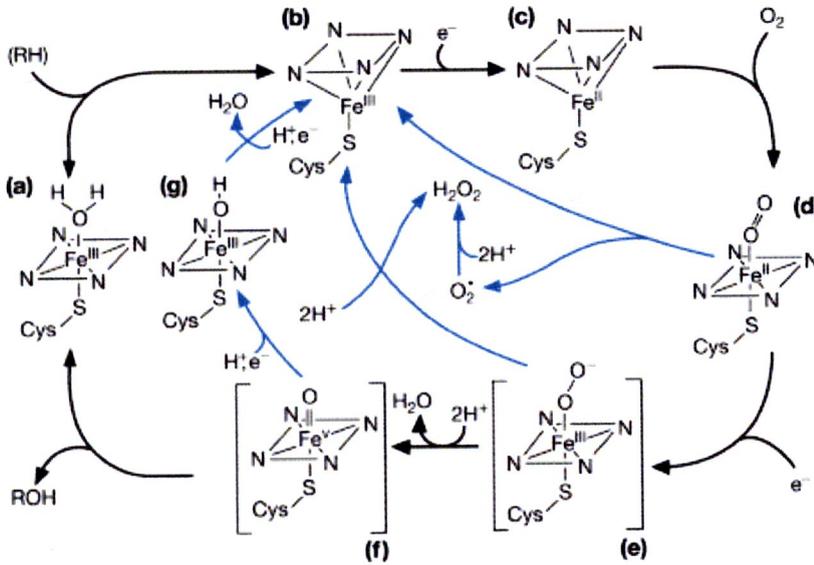


Fig 2.4A scheme of the catalytic cycle of cytochrome P450 [39]

In the substrate-free form, most CYPs have their heme iron predominately represented by its six-liganded low-spin state (a). The binding of the substrate shifts the spin equilibrium towards the free-coordinated high-spin form. After acceptance of the first electron, the ferrous CYP binds an oxygen molecule. The oxy-complex of CYP (d) is further reduced to give a peroxy-complex (e), protons input, can result in the heterolytic cleavage of the O-O bond producing water and the oxenoid complex (f). The heme-bound activated oxygen atom is inserted into the substrate. In the presence of a high concentration of hydrogenperoxide or organic peroxide, these molecules may serve as a source of oxygen for CYP and the enzyme may catalyze

hydrogen peroxide- dependent substrate hydroxylation in the truncated catalytic cycle as shown in gray arrow.

Blue arrows show “leaky”, ROS- producing branches. The first branch leading to the production of ROS is the release of superoxide anion radical due to decay of the one-electron-reduced ternary complex (d). The second ROS- producing branch is the reverse of the peroxide shunt that is the protonation of the peroxycytochrome P450 (f) with formation of hydrogen peroxide. The third leaky branch requiring the addition of two more electrons following the decay of the peroxycytochrome P450 (e) resulting in the release of the second oxygen atom in a water molecules. Continuous production of ROS is an inevitable result of NADPH consumption by CYP microsome both in the presence and absence of substrate. However, the partition of these branches depends on many conditions for example, the substrates, pH, ionic strength and oxygen concentration [48].

Role of CYP-generated ROS in physiology and disease

ROS are important cell-signaling pathway mediators and important role in many disease and aging [49]. Although many enzymes can generate ROS, CYP enzymes have important role in generate them. It has been proposed that CYP is important sources of catalytic iron undergoes redox cycling and contribute to toxicity. Tissue injury can generate oxygen radicals and increase in catalytic iron. The presence of CYP substrates during reperfusion blocks the increase in catalytic iron and associated tissue injury. Many hepatic CYPs are down regulated in response to inflammation. In humans, this down regulation may have clinically important effects

on drug metabolism. Studies with endotoxin and nitric oxide synthase inhibitors have suggested that CYPs may be down regulated at the gene transcription level, mRNA stability, and enzyme activity [50]. CYP2E1 is believed to contribute to alcoholic liver disease and nonalcoholic steatohepatitis through effects on lipid hydroxylation [51]. One mechanism by which hepatic fibrosis may be mediated in liver disease is through stimulation of collagen production in stellate cells. Although ethanol treatment increase total levels of hepatic CYP2E1 and thereby increase the amount of ROS generated by CYP2E1, ethanol itself does not directly affect CYP2E1 uncoupling link between CYP2E1 protein levels, hydrogen peroxide formation and cisplatin nephrotoxicity. [52].

2.4.2 Phase II enzymes

2.4.2.1 *Uridine diphosphate glucuronosyltransferase*

Glucuronidation is the major and important sugar conjugation pathway in animal kingdom. Glucuronide formation is catalyzed by uridine diphosphate glucuronosyl transferase (UGT) [EC 2.4.1.17]. UGT can react with endogenous compounds such as bilirubin, steroids and thyroid hormone into more hydrophilic and easy excretable metabolite by transferring glucuronic acid residue from uridine diphosphate glucuronic acid (UDPGA) to substances [53]. UGTs play an important cytoprotective role, either by preventing the accumulation of potentially toxic xenobiotics or by avoiding their subsequent bioactivation to even more toxic reactive intermediates. A wide variety of compounds can be substrates for glucuronidation. Four general categories of *O*-, *S*-, *N*- and *C*- glycosides have been reported as shown

in Fig 2.5. Xenobiotic glucuronidation occurs abundantly in the liver, intestine mucosa, and kidney. Almost all other organs and tissue possess some glucuronidation activity but at very low levels. UGT enzymes are the products of multigene superfamily. Human UGT's identified to date consist of three gene families UGT1, UGT2 and UGT8 and members of the families UGT1 and UGT2 contribute to xenobiotics metabolism. The human UGT1 family consists of at least 13 enzymes that are the products of alternate splicing of single gene. This gene contains 13 known promoters/first exons that can splice to the common exons 2 through 5, producing 13 different products. Some UGT1 family members are inducible by aryl hydrocarbon receptor (AhR) ligands, constitutive androstane receptor (CAR) receptor ligands and pregnane X receptor (PXR) receptor ligand. UGT1 family members are responsible for bilirubin and thyroxine conjugation as well as the conjugation of some xenobiotics. Human UGT2 family consists of at least five members that are all within the UGT2B subfamily. Some of the UGT2 family members are susceptible to induction by CAR receptor ligands. The UGT2 enzymes are largely responsible for glucuronide conjugate of steroid hormones and some xenobiotic including 1-naphthol, 4-nitrophenol, 4-hydroxybiphenyl [54, 55].



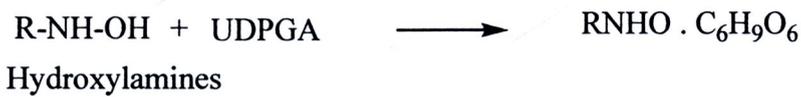
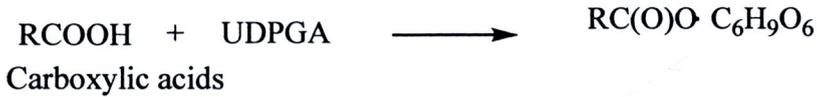
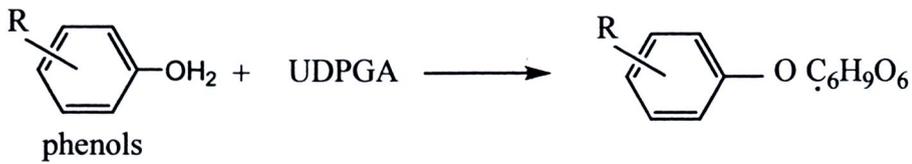
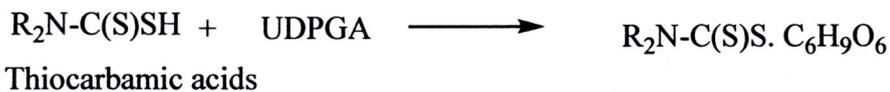
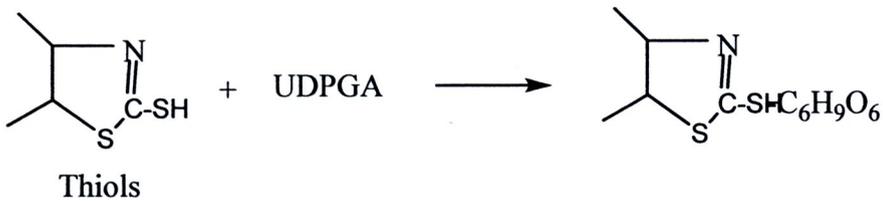
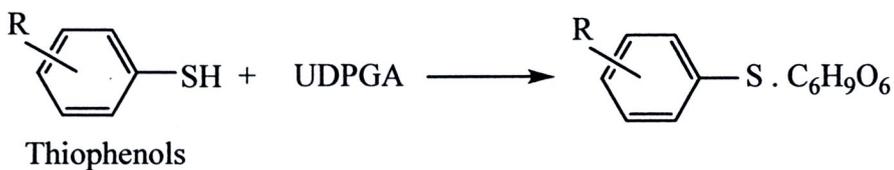
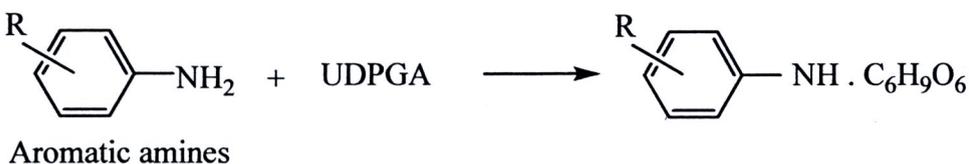
O-Glucuronide**S-Glucuronides****N-Glucuronides**

Fig.2.5 Types of glucuronides formed by UGT enzymes [55]

2.4.2.2 *Glutathione S-transferase*

Glutathione S-transferase (GSTs; E.C.2.5.1.18) are multi gene family enzymes involved in the detoxification of a widely chemicals [57]. GST catalyze the nucleophilic attack of glutathione (GSH) on electrophilic substrates, thereby decreasing their reactivity with cellular macromolecule. Most GSTs exist as soluble enzymes, although a small family of microsomal GSTs has been characterized and a mitochondrial GST, referred to as GST kappa, has also been soluble GSTs are collectively expressed in rather large amounts, constituting as much as 45% of total soluble protein in the liver. GSTs catalyze the general reaction:



The function of the enzyme is to bring the substrate into close proximity with glutathione (GSH) by binding both GSH and the electrophilic substrate to the active site of the protein and activate the sulhydryl group on GSH, thereby allowing for nucleophilic attack of GSH on the electrophilic substrate (R-X). Substrates for GST including, environmental toxicants, pesticides, drugs and endogenous molecule, for example, benzo(a)pyrene 7,8-dihydrodiol-9, 10- epoxide, styrene oxide, ethylene oxide, lindane, DDT, methyl parathion, chlorambucil, thiopeta, nitroglycerine and acetaminophen. The electrophilic functional center of the substrates can be a carbon, nitrogen or sulfur. The formation of a thioether bond between the cysteine residue of GSH and the electrophile usually results in a less reactive and more water soluble product, and thus GST are usually detoxification reaction [58].

2.4.2.3 Heme oxygenase

Heme oxygenase (HO) is the first, and the rate limiting enzyme in the heme catabolism pathway to biliverdin, carbon monoxide (CO) and free iron. Three isoforms of HO designated as HO-1, HO-2 and HO-3 have been identified in mammals. These are products of different in gene, their expression, tissue distribution and regulation. HO is expressed in prokaryotic cells and eukaryotic cells, regulating a wide spectrum of cellular processes. HO-1, known as heat shock protein 32, is strongly induced by hypoxia, hydrogen peroxide, depletion of cellular glutathione and heme. HO-1 plays a vital function in maintaining cellular heme homeostasis in addition to heme degradation. HO-1 induction protects cells from ischemia/reperfusion injury, oxidative stress, inflammation, transplant rejection, and apoptosis. HO-1 functions as a stress-responsive anti-oxidant enzyme, with the capacity to protect against oxidant-mediated vascular injury. HO-2 and HO-3 isoforms probably function normally as first defense against oxidative/ inflammatory insults. HO-2 is highly expressed in brain and testis and protects neurons against oxidative stress. Overexpression of the HO-1 gene provides cytoprotection against oxidative stress, the specific activation of HO-1 gene expression by pharmacological modulation represent a novel target for therapeutic intervention [59].