CHAPTER 1

INTRODUCTION

1.1 Statement of the problems

Cancer is a major chronic disease worldwide. About 90–95% of all cancer cases are caused from environment and lifestyle, especially diet, whereas the remaining 5–10% can be attributed to genetic defects (Anand *et al.*, 2008). From these reasons, cancer is a preventable disease by changing diet and lifestyle. The initiation stage, which involves the induction of mutation, is widely recognized as an important step in chemical carcinogenesis. Carcinogens can covalently bind to DNA, especially protooncogenes and tumor suppressor genes, forming DNA-adduct which lead to initiated or mutated cells. (Goldman and Shields, 2003)

Epidemiological studies have found high intakes of vegetables and fruits are associated with a low rate of cancer in humans. Plant flavonoids have many biological and pharmacological properties including anticancer activity. Flavonoids may act at different stages of carcinogenesis. Their antitumor mechanisms include antioxidant activity, inactivation of carcinogens, antiproliferation, induction of apoptosis, blocking of the cell cycle and inhibition of angiogenesis. With multidirectional action in carcinogenesis, it is suggested that flavonoids may give a new perspective of their use in prevention of cancer (Majewska-Wierzbicka and Czeczot, 2012).

Rice (*Oryza sativa* L.) is an important agricultural product and a main staple food in Thailand. Rice is rich in many nutrients including carbohydrates, proteins, certain fatty acids, trace minerals and vitamins especially vitamin E. It is also a source of many bioactive non-nutrient compounds such as γ-oryzanol, phenolic acids and flavonoids (Huang and Ng, 2011; Vichapong *et al.*, 2010). Interestingly, glutinous purple rice *(Oryza sativa* L.var.*indica)* variety Kum Doisaket, cultivated in northern Thailand, has the purple color of the hull and pericarp. It contains high amount of

flavonoids, including anthocyanins that have several pharmacological and biological properties. Previous studies have demonstrated that Kum Doisaket has the highest values for total phenolic, cyanidin-3-glucoside and γ-oryzanol contents when compared to other varieties of purple rice (Boonsit *et al.*, 2010). It also showed *in vitro* antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays (Daiponmak *et al.*, 2010). Our previous study found the methanol extract of glutinous purple rice seed and the dichloromethane extract of glutinous purple rice hull presented the highest antimutagenicity using Salmonella mutation assay. Futhermore, acidified methanol extract of glutinous purple rice hull contained the highest amounts of total phenolic, total flavonoids and cyanidin-3-glucoside. However, it showed both strong antimutagenicity and potent mutagenicity in Salmonella mutation assay in either presence or absence metabolic activation (Punvittayagul *et al.*, 2011).

In this study, we evaluated clastogenicity and anticlastogenicity of glutinous purple rice *(Oryza sativa* L.var. *indica)* extracts, the methanol extract of rice seed, the dichloromethane and acidified methanol extracts of rice hull, using rat liver micronucleus as the end-point biomarker. The possible inhibitory mechanism of glutinous purple rice extracts on an initiation stage of hepatocarcinogenesis involving xenobiotic metabolizing enzymes was evaluated.

1.2 Literature reviews

1.2.1 The role of chemicals in mutagenesis related to carcinogenesis

Cancer is a disease caused from the accumulation of either spontanous or chemical induced mutation (Pray, 2008). These chemicals are classified into genotoxic and non-genotoxic carcinogens. Genotoxic carcinogens cause DNA damage through several mechanisms such as point mutation, deletion and insertions, gene recombinations, gene rearrangements and gene amplifications, as well as chromosomal aberrations. Nongenotoxic carcinogens do not produce mutation in DNA but they alter the expression of certain genes and/or alter pathways that influene cellular events related to proliferation, differentiation or apoptosis (Sutandyo, 2010).

Carcinogenesis is a multi-step process, composing of initiation, promotion and progression (Figure 1-1).

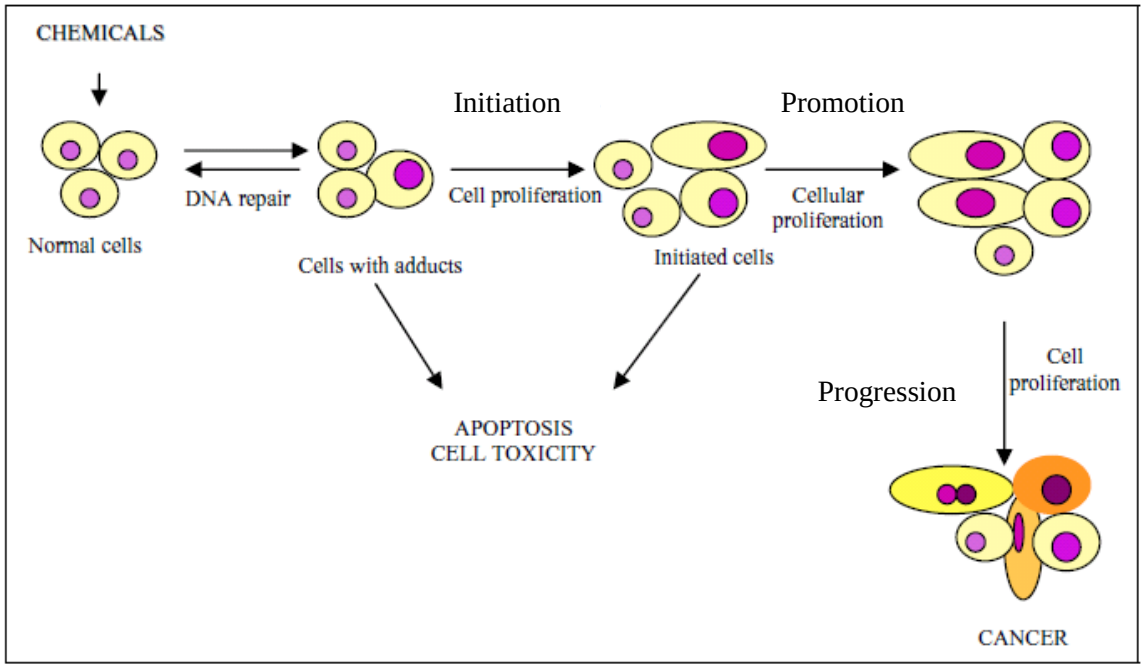

Figure 1-1 Chemical carcinogenesis stages (Oliveira *et al.*, 2007)

Initiation is the first stage of the cancer process. This stage is a rapid and irreversible process that result in a carcinogen-induced mutation. Carcinogens can covalently bind to DNA and form adducts resulting DNA mutation. The initiating event becomes fixed when the DNA damage is not correctly or completely repaired prior to DNA synthesis. Once initiated cells are formed, there are at least 3 potential outcomes. The first, the initiated cell can remain in a static non-dividing state. The second, the initiated cell may be deleted though apoptotic mechanisms. The last, the cell may undergo cell division resulting in the proliferation of the initiated cell.

Promotion stage involves the selective clonal expansion of initiated cells to produce a preneoplastic lesion. Both exogenous and endogenous agents that operate at this stage are refered to as tumor promotors. Tumor promotors act through several mechanisms involving gene expression changes that result in sustained cell proliferation through increase in cell proliferation and/or the inhibition of apoptosis. Promotion is reversible on removal of promoting agent and the focal cells can return to be a single initiated cell. In addition, these agents demonstrate a well-documented threshold for their effects. Below a certain dose or frequency of application, tumor promoters are unable to induce cell proliferation. Tumor promoters generally show organ-specific effects, for example, a tumor promoter of the liver such as phenobarbital will not function as a tumor promotor in the skin or other tissues.

Progression involves the conversion of benign preneoplastic lesions into neoplastic cancer. In this stage, due to increase in DNA synthesis and cell proliferation in the preneoplastic lesion, additional genotoxic events may occur resulting in further DNA damage including chromosome aberration and translocation. The progression stage is irreversible in that neoplasm formation, whether benign or malignant, occurs. With the formation of neoplastic, an autonomous growth and/or lack of growth control is achieved. Spontaneous progression can occur from spontaneous karyotypic changes that occur in mitotically active initiated cells during promotion. An accumulation of nonrandom chromosomal aberrations and karyotypic instability are hallmarks of progression (Klaassen and Watkins, 2010).

1.2.2 Micronucleus assay

The *in vivo* micronucleus assay is the primary test in a battery of genotoxicity which detects both clastogenicity (chromosome breakage) and aneugenicity (chromosome lagging due to dysfunction mitotic apparatus). Micronucleus mainly originates from aneugen which increase chromosome missegregation. The chromosomes that break after anaphase can be surrounded by the nuclear envelope, forming micronuclei (Figure 1-2A). Figure 1-2B shows clastogens induce chromosome breaks that yield acentric fragments which are easily included into micronuclei. Double minutes (DMs) are autonomously replicating acentric chromatin bodies composed of circular DNA that do not require a telomeric end and contain highly amplified genes (Figure 1-2C). Moreover, chromosomal instability at mitosis are closely related to the breakage-fusion-bridge. The bridge breakage often results in the formation of acentric fragments that are not included in any of the daughter cell nuclei and form one or more micronuclei at the end of mitosis. The dicentric chromosomes involved in anaphase bridges are sometimes detached from the two centrosomes, left behind at anaphase and sequestered into micronuclei (Figure 1-2D) (Traradas *et.al.,* 2010).

The rodent micronucleus assays can be detected in bone marrow, liver, alveolar, bladder, buccal mucosa, colon, skin and neonatal tissues (Morita *et al*., 2011). The bone marrow micronucleus test is the most common assay, but some compounds such as unstable mutagens or those which generate short-lived metabolites, are not detected in this test because the metabolites produced in the liver do not reach the bone marrow (Cliet *et al*., 1989). The use of hepatocytes in rat liver has several advantages. (I) The broad spectrum of metabolizing enzymes expressed in hepatocytes ensures an adequate activation of most xenobiotics. (II) Hepatocytes are target cells of special interest when compounds are investigated which act specifically in the liver. Especially for hepatocarcinogens classified as nongenotoxins in standard genotoxicity tests or for chemicals showing DNA-repair induction in hepatocytes but no mutagenicity in standard tests. (III) The hepatocyte micronucleus assay can be performed following an *in vivo* or *in vitro* with the high reliability and low cost (Muller-tegethoff *et al.,* 1995).

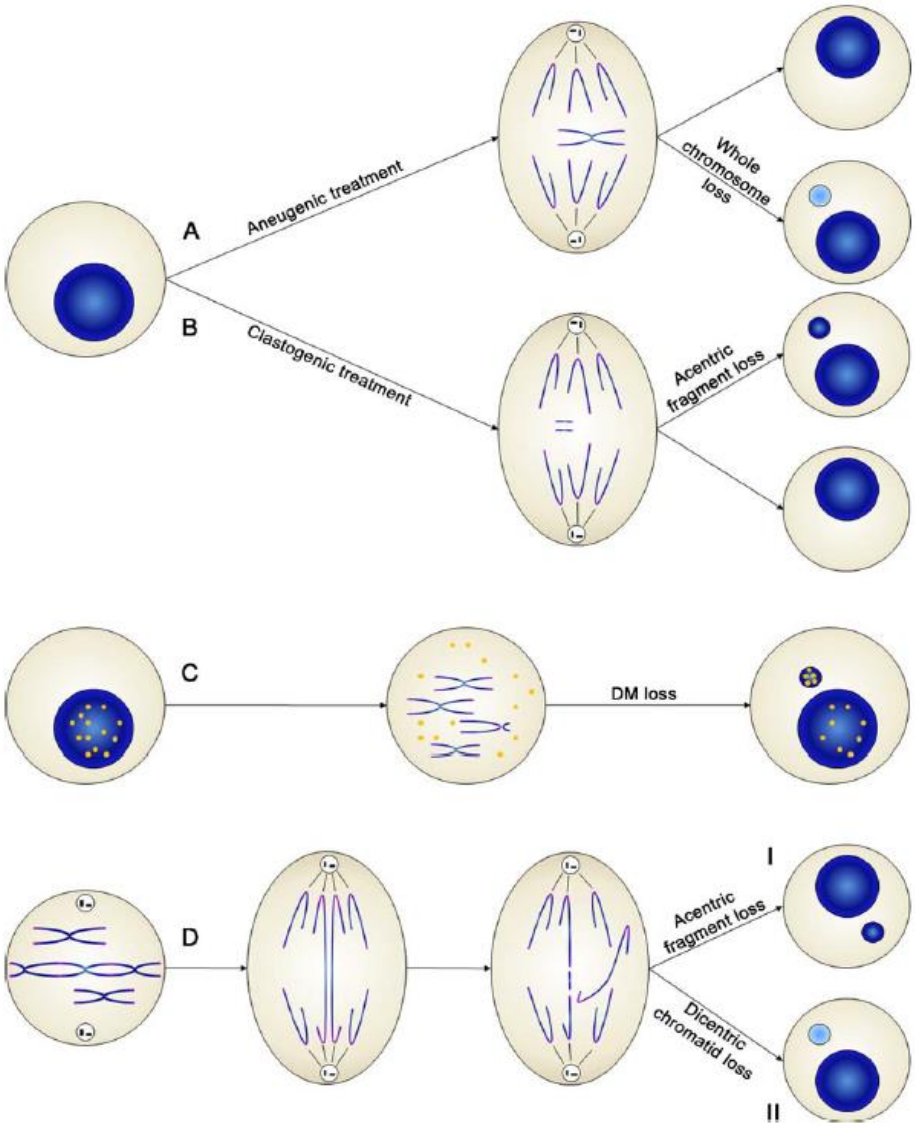

Figure 1-2 Mechanisms of micronuclei formation (Traradas *et al.*, 2010)

1.2.3. Xenobiotic metabolism

Xenobiotics are chemical substances that are foreign to living cells. They includes such examples as drugs, pesticides, cosmetics, flavorings, fragrances, food additives, industrial chemicals and environmental pollutants (Patterson *et al.*, 2010). Most of these chemicals enter to the body via the diet, air, drinking water, drug administration, and lifestyle choices then distributed across several tissues reaching the liver at the later stage excretion by xenobiotic metabolizing enzymes. These enzymes generally render xenobiotics, more polarity and easily excretable. The reactions of xenobiotic metabolism composed phase I and II (Oliveira *et al*, 2007).

Phase I reactions include transformation of a parent compound to more polar metabolite by *de novo* formation of functional groups such as -OH, -NH_2, -SH. The examples of reactions include *N*- and *O*-dealkylation, aliphatic and aromatic hydroxylation, *N*- and *S*-oxidation, and deamination. The main enzymes in this phase are cytochrome P450s (CYP450s) acting as monooxygenases, dioxygenases and hydrolases (Anzenbacher and Anzenbacherova, 2001).

Phase II enzymes play a role in the biotransformation of endogenous compounds and xenobiotics to more easily excretable forms as well as in the metabolic inactivation of active substances. The purpose of phase II biotransformation is to perform conjugating reactions. These include glucuronidation, sulfation, methylation, acetylation, glutathione and amino acid conjugation. In general, the respective conjugates are more hydrophilic than the parent compounds. Phase II drug metabolizing enzymes are mostly transferases including sulfotransferases, UDP-glucuronosyltransferases, *N*-acetyltransferases, glutathione *S*-transferases and methyltransferases and catechol *O*-methyl transferase (Janacova *et al.*, 2010).

Phase I and II enzymes can be induced by numerous chemicals. There are 2 types of enzyme inducers: monofunction and bifunction (Figure 1-3). Bifunctional inducers increase phase II enzymes as well as phase I enzymes, such as aryl hydrocarbon hydroxylase, and bind with high affinity to the aryl hydrocarbon receptor xenobiotic response element (Sogawa and Fujii-Kuriyama, 1997). Monofunctional inducers induce phase II enzymes selectively and will activate the antioxidant response element (ARE) through Keap1 and Nrf2 (Dinkova *et al*.,2002).

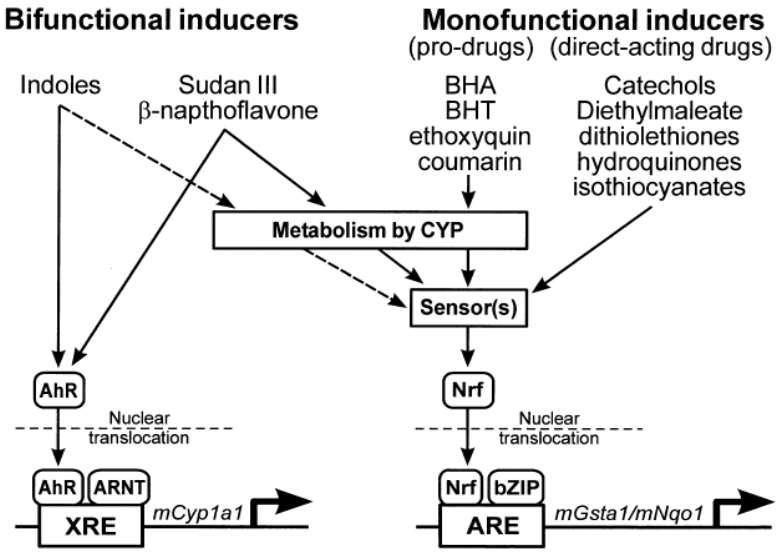

Figure 1-3 Mechanism of enzyme inducers (Hayes and Mcmahon, 2001)

1.2.3.1 Cytochrome P450s

The cytochrome P450s are hemoproteins that play critical roles in the bioactivation and detoxification of a variety of xenobiotic substances. In mammalian cells, P450s are localized predominantly in smooth endoplasmic recticulum of cell. They are entirely distinct from the cytochrome proteins of electron transport system in mitochondria. Cytochrome P450s include several families which are distinguished by the xenobiotic substrates activated and metabolized, as well as by their inhibitors and inducers (Omiecinski *et al*.,1999). For example, among the three major P450 forms in human liver microsomes, cytochrome P450 isoform 3A4, presumably with a large substrate-binding pocket, catalyzes the biotransformation of many drugs. Cytochrome P450 isoform 2E1 with apparently a small substrate-binding pocket catalyzes the oxidation of many volatile environmental chemicals and anesthetics. Cytochrome P450 isoform 1A2 catalyzes the activation of carcinogenic arylamines and aflatoxin B1 (Yang *et al*., 1994).

1.2.3.2 NADPH-cytochrome P450 reductase

NADPH-cytochrome P450 reductase (CPR) is the electron donor protein for several oxygenase enzymes found on the endoplasmic reticulum of most eukaryotic cells. CPR is a flavoprotein containing both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). FAD is the electron acceptor flavin from NADPH and that FMN is the electron donor to acceptor proteins such as the cytochromes P450 (Figure 1-4), heme oxygenase-1, cytochrome *b5*, 7-dehydrocholesterol reductase and squalene monooxygenase (Mizutani and Ohta, 1998).

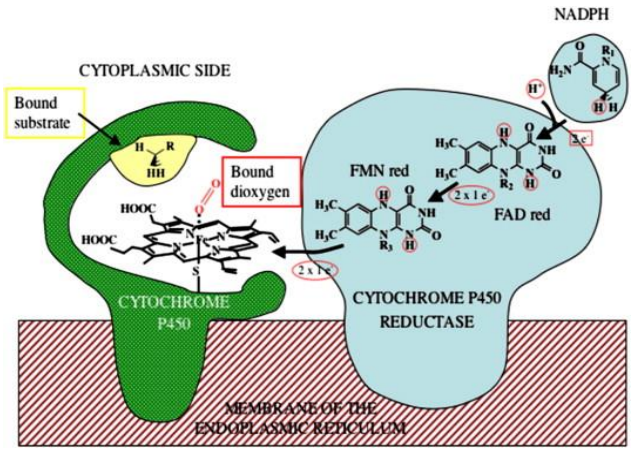

Figure 1-4 Function of cytochrome P450–cytochrome P450 reductase complex (Mizutani and Ohta, 1998)

1.2.3.3 Heme oxygenase

Heme oxygenase (HO) is a microsomal enzyme catalyzing the first, rate-limiting step in degradation of heme and playing an important role in recycling of iron. It cleaves the α-meso carbon bridge of heme, yielding equimolar quantities of carbon monoxide (CO) and ferrous (Fe^{2+}) and biliverdin. CO is then exhaled from the organisms through the lung. Free iron induces the expression of the iron-sequestering ferritin and activates Fe-ATPase, an iron transporter, which decrease intracellular Fe^{2+} content. Finally, biliverdin is converted by biliverdin reductase to bilirubin, which can be oxidized by cytochrome P450 enzymes or glucoronidated by UDP-glucuronyl-transferase and subsequently eliminated as bilirubin glucoronides by the biliary-fecal pathway (Gozzelino *et al*., 2010).

Three distinct mammalian HO isoforms have been identified. HO-1, the inducible 32-kDa isoform, is highly expressed in the liver and spleen, but can be also detected in many other tissues. HO-2 is a constitutively expressed 36-kDa protein, presents in high levels in the brain, testes, or endothelial cells. HO-3 is postulated as a 33-kDa protein expressed in different organs, very similar to HO-2, but with much lower catalytic activity (Zhu *et al*., 2010). HO results in decreased oxidative stress, attenuated inflammatory response and reduced apoptosis (Figure 1-5). CO, product of this reaction, is an important cellular messenger, with the signaling function resembling that of nitric oxide. CO induces soluble guanylyl cyclase and thereby inhibits platelet aggregation, decreases leukocyte adhesion, and reduces endothelial cell apoptosis. In addition, it exerts antiinflammatory effects by inhibition of tumor necrosis factor, interleukin-1β , and macrophage inflammatory protein-1β, or by up-regulation of interleukin-10. Ferrous iron, the second product of heme decomposition, can be potentially toxic, giving rise to hydroxyl free radicals. Simultaneous up-regulation of ferritin and cytosolic iron efflux, however, protects cells from oxidative stress. Both biliverdin and bilirubin, which have been long regarded as toxic end products of heme metabolism, are inhibitors of the complement cascade and potent antioxidants, capable of reducing the inflammatory response and attenuating oxidative injury by scavenging peroxyl radicals and decreasing peroxidation of membrane lipids and proteins (Jozkowicz *et al.*, 2007).

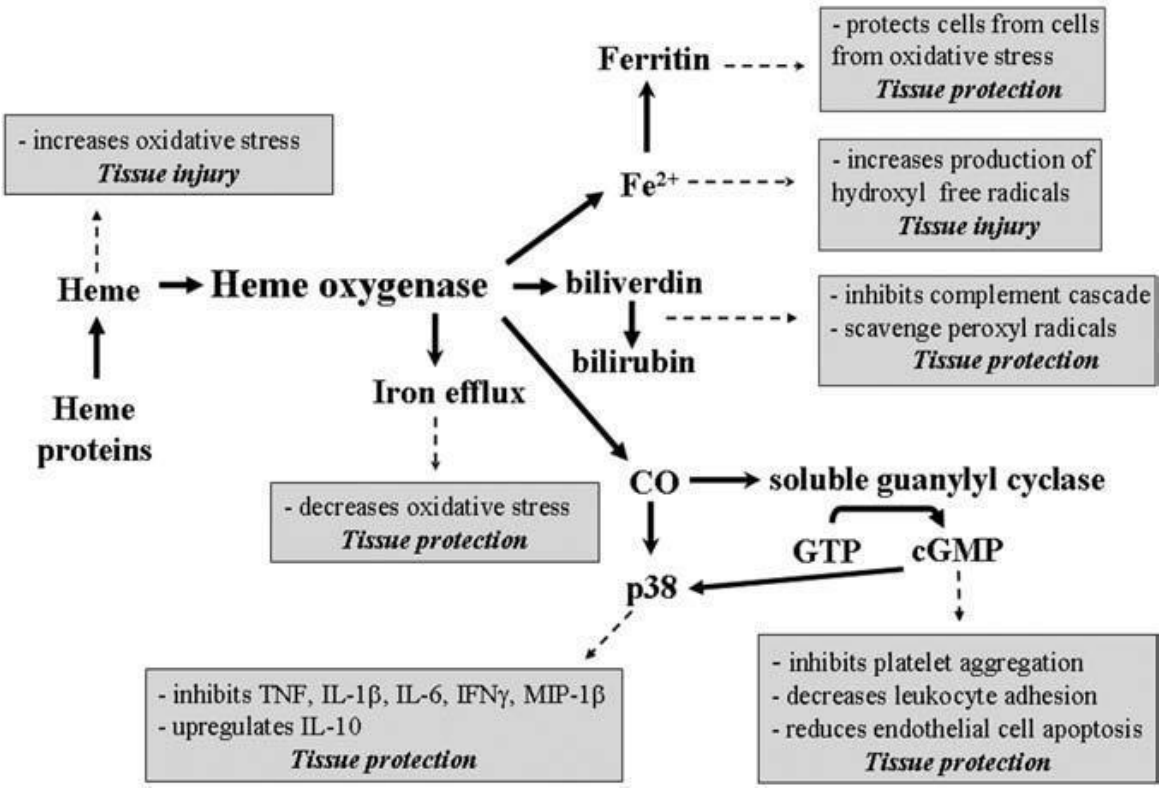

Figure 1-5 Heme oxygenase pathway and its biological activities (Jozkowicz *et al.*, 2007)

1.2.3.4 Glutathione *S*-transferases

Glutathione *S*-transferases (GSTs) are a family of enzymes that catalyze the formation of thioether conjugates between the endogenous tripeptide glutathione and xenobiotic compounds (Figure 1-6). GSTs play a major role in the detoxication of epoxides derived from polycyclic aromatic hydrocarbons and alpha-beta unsaturated ketones. Moreover, a number of endogenous compounds such as prostaglandins and steroids are metabolized via glutathione conjugation (Van Bladeren, 2000).

The major biological function of glutathione *S*-transferases appears to be defense against reactive and toxic electrophiles such as reactive oxygen species (superoxide radical and hydrogen peroxide) that arise through normal metabolic processes. Many of these are formed by cellular oxidative reactions catalyzed by cytochrome P450s and other oxidases (Sheehan *et al.*, 2001).

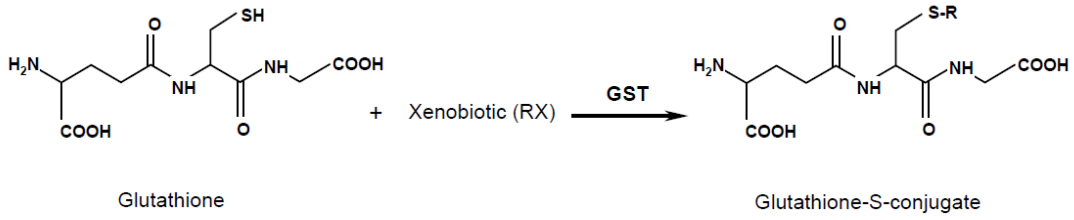

Figure 1-6 Formation of glutathione conjugate (Van Bladeren, 2000)

1.2.3.5 UDP-glucuronyltransferases

UDP-glucuronosyltransferases (UGT) are a superfamily of membrane-bound enzymes catalyzing the formation of a chemical bond between a nucleophilic O-, N-, S-, or C-atom with uridine-5′-diphospho-α-D-glucuronic acid. The formation of β-D-glucuronide conjugates is the most important detoxication pathway of the Phase II of drug metabolism in all vertebrates (Figure 1-7) with easy elimination via bile or urine. In humans, approximately 40–70% of all clinical drugs are subjected to glucuronidation reactions metabolized by UGT (Wells *et al*., 2004).

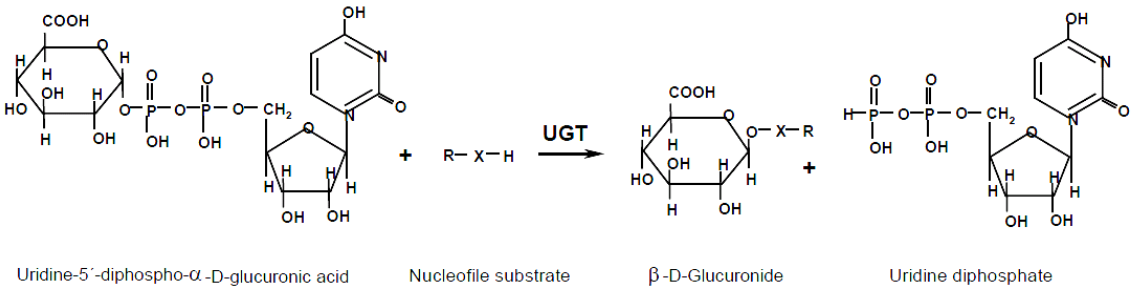

Figure 1-7 Conjugation of a nucleophile substrate with uridine-5'-diphospho-α-D-glucuronic acid (Jancova *et al.*, 2010)

1.2.3.6 NAD(P)H:quinone oxidoreductase-1

Quinones are found in plants, fungi and bacteria. Human exposes to quinones via the diet, drugs and airborne pollutants (Monks and Jones, 2002). Quinones are highly redox active molecules which can redox cycle with their semiquinone radicals, leading to formation of reactive oxygen species, associated with aging and carcinogenesis (Bolton *et al.*, 2000).

Quinone can be metabolite by two pathways (Figure 1-8). Fully oxidised quinones by cytochrome P450s reductase can readily undergo single-electron reduction reactions. This results in production of reactive semiquinone intermediates. These molecules can form adducts directly with cellular macromolecules including DNA, and are thereby carcinogenic. NAD(P)H:quinone oxidoreductase-1 (NQO1) is an alternative enzyme which can protects against the deleterious effects of quinones by catalysing their two-electron reduction, in a single stage, thus by-passing the semiquinone intermediate. The resulting hydroquinone is relatively stable and can readily be further conjugated and excreted. Many studies show that NQO1 protects against the toxicity and carcinogenicity of quinones (Nioi and Hayes, 2004).

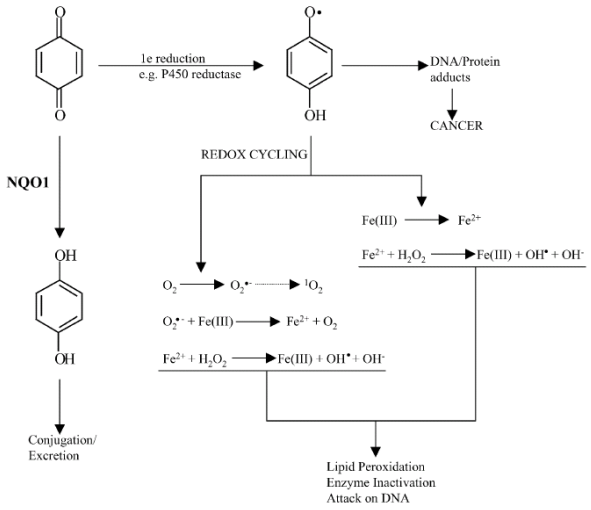

Figure 1-8 Quinone metabolism (Nioi and Hayes, 2004)

1.2.4 Diethylnitrosamine induced hepatocarcinogenesis

N-Nitroso compounds represent a well-established class of chemical carcinogens. Human exposure to preformed N-nitrosamines occurs through the diet, in certain occupational settings, and through the use of tobacco products, cosmetics, pharmaceuticals, and agricultural chemicals. In addition, N-nitrosamines are generated in the body by nitrosation of amines. Both nitrate and nitrite are capable to form nitrosamines, a large group of compounds with common carcinogenic mechanism. Humans are exposed to N-nitroso compounds in diet from a variety of cured meats and fish products. Sodium nitrite has been used as food addictive for preservation and as coloring substance in meat (Fiddler, 1975).

N-Nitrosamines require metabolic activation to form DNA adducts that are critical for their mutagenic and carcinogenic activities. The major pathway is R-hydroxylation (adjacent to the N-nitroso group), catalyzed by cytochrome P450 isoform 2E1 (Figure 1-9). The common carcinogenic mechanism of N-nitrosamines is associated with formation of N-nitrosodiethylamine, which undergoes enzymatic hydroxylation and subsequent hydrolysis to aldehyde and monoalkylnitrosamide that rearranges and releases a carbocation that is reactive adduct at N- and O- atom in DNA bases, especially O^6-methylguanine leading to GC-AT transition (Verna *et al.*,1996).

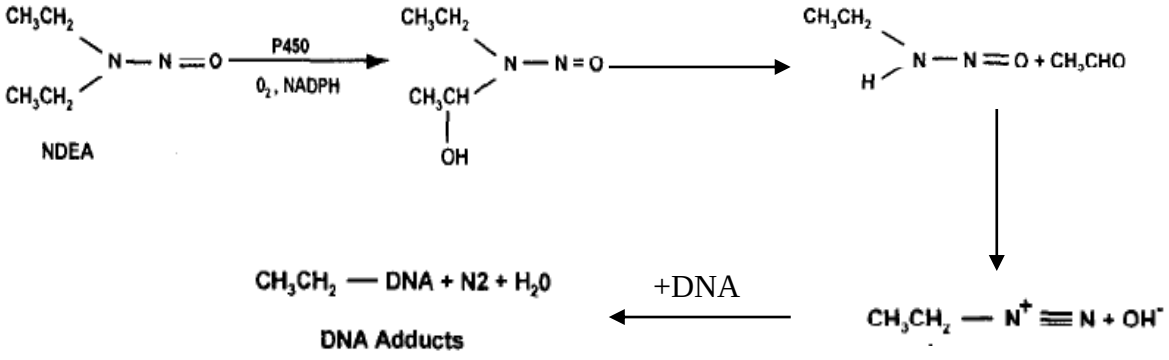

Figure 1-9 Biotransformation of diethylnitrosamine and mechanism of DNA-adduct formation (Verna *et al.*, 1996)

Since, DEN leads to the initiation of hepatocytes or to the death of augmenting the ability of those cells that have DEN-induced oncogenic mutations to generate transformed progeny. Such increased proliferation is then followed by dysplasia, adenoma, and hepatocellular carcinoma formation (Vakkila and Lotze, 2004). More cellular and molecular basis of DEN-induced hepatocarcinogenesis has been proposed. Liver injury in response to DEN exposure elicits an inflammatory response in nonparenchymal cells (NPCs), such as Kupffer cells and stellate cells (Figure 1-10). NPCs secrete NF-κB-regulated hepatomitogens such as TNF-α, IL-4, and HGF, which promote compensatory proliferation of DEN-induced mutated hepatocytes. This process allows for the transmission of genetic alterations to daughter cells, thereby favoring liver neoplastic progression. Alternatively, autocrine secretion of TGF-α by hepatocytes induces cell survival and proliferation in the absence of liver damage and independent of NPCs-mediated secretion of hepatomitogens (Maeda *et al.*, 2005).

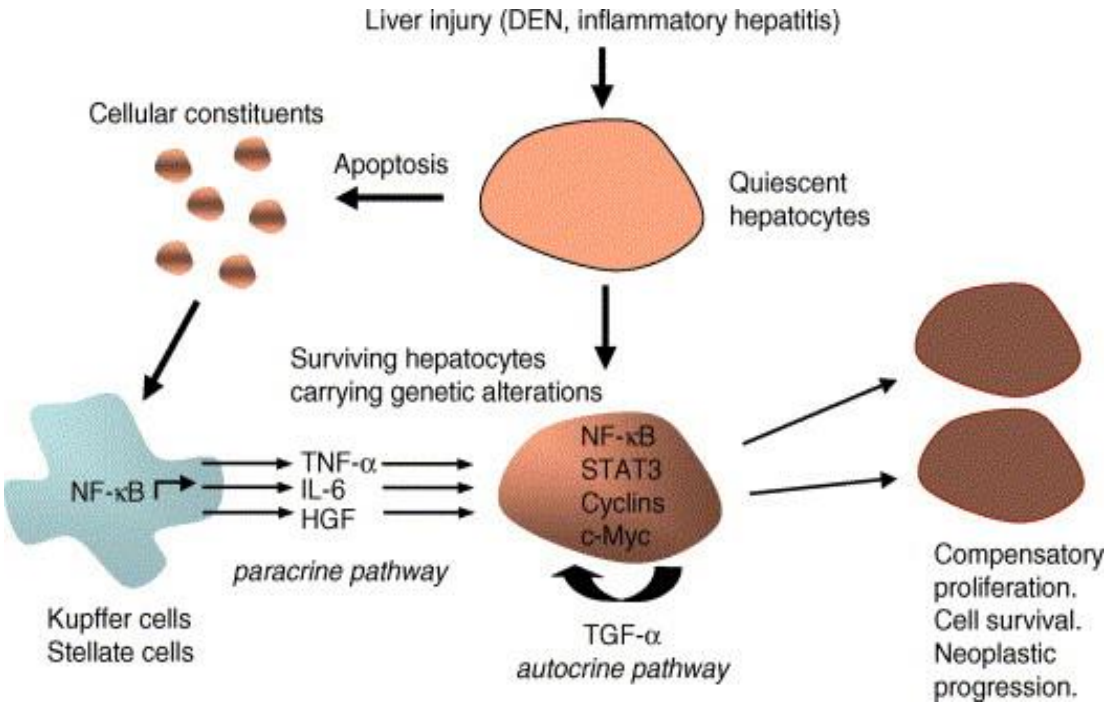

Figure 1-10 Diethylnitrosamine induced proinflammatory cytokines production (Arsura and Cavin, 2005)

1.2.5 Cancer chemoprevention

Cancer prevention can be categorized as primary and secondary prevention. Primary prevention refers to the identification of genetic, biological and environmental factors that play an etiologic or pathogenetic role in order to impair their effects on tumor development and halt progression of cancer and ultimately death. The objective of primary prevention is to prohibit or to halt effective contact of a carcinogenic agent with a susceptible target in the human body. Secondary prevention refers to identification of existing pre-neoplastic and early neoplastic lesions in order to treat them thoroughly and expeditiously. Since the stage of cancer dictates the therapeutic choice, early detection is a primary objective. The goal of cancer screening is to reduce mortality through a reduction in incidence of advanced disease (Cabibbo *et al.* ,2012).

Many natural dietary constituents from plants can be considered as chemopreventive agents. Chemopreventive agents have been divided into two broad groups. Compounds that prevent mutagenesis have been termed blocking agents and compounds that act post-mutagenesis have been termed suppressing agents (Chen and Kong, 2004) (Figure 1-11). Blocking agents are typically those compounds that can inhibit initiation either by inhibiting the formation of carcinogens from precursor molecules or reactive metabolites from the parent carcinogens, or by preventing the ultimate electrophilic and carcinogenic species from interacting with critical cellular target molecules, such as DNA, RNA, and proteins and can stimulate the detoxification of carcinogens, leading to their secretion from the body. Moreover, cancer initiation can be inhibited by triggering protective mechanisms either in the extracellular environment or inside cells including inibition of uptake of mutagens or carcinogen, inhibit the endogenous formation of mutagen or cacinogen, modification of transmembrane transport, modulate of metabolism, maintenance of DNA structure, modulation of DNA repair and control of gene expression. Suppressing agents are considered to inhibit malignant expression of initiated cells, in either the promotion or the progression stage via inhibit imflammatory activity, cell proliferation, modulation cell apoptosis, inhibit angiogenesis, effect on cell-adhesion molecules and activation of antimetastasis genes (Ferguson *et al.*, 2005; Surh, 2003).

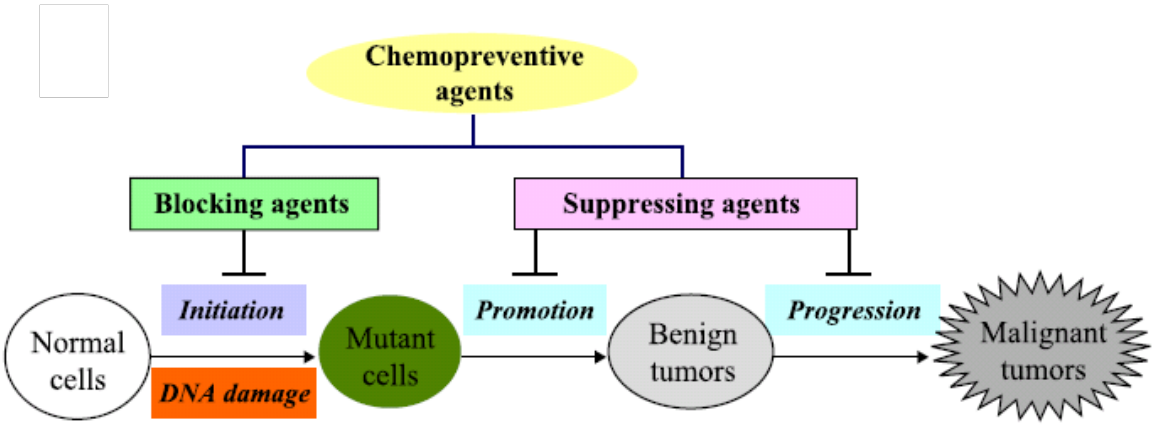

Figure 1-11 Concept of cancer chemoprevention (Chen and Kong, 2004)

1.2.6 Pharmacological and biological effects of rice

Rice (*Oryza sativa* L.) is the most important cereal crop in the world, either directly as human foods or indirectly as animal feeds. It is the staple food of over half the world's population. Rice is rich in many nutrients including both macronutrients and micronutrients. Rice is also a source of numerous bioactive non-nutrient compounds. The common phenolic compounds in whole grains are phenolic acids and flavonoids. These phenolic acids are ferulic acid, vanillic acid, caffeic acid, syringic acid and *p*-coumaric acid while flavonoids are flavonols, flavan-3-ols, flavones and flavanones (Vichapong *et al.*, 2010)

Rice hull is a low-value waste product, yet contains many bioactive ingredients. Ferulic and *p*-coumaric acids are the major phenolic acids in rice hull (Butsat and Siriamornpun, 2010). Ferulic acid has a potential anti-carcinogenic properties on the UVB induced epidermic tumor development by blocking the relevant cytokine secretion and expression of p53, p21, c-fos, PCNA and RPA genes (Lin *et al.*, 2010). The *p*-coumaric acid possesses potent anticancer properties due to the inhibition of angiogenesis by suppressing both the AKT and ERK signaling pathways, which are known to be crucial for angiogenesis (Kong *et al.*, 2012). Previous study has reported that rice hull extract exhibited high antioxidant activity against scavengers of singlet oxygen and inhibited high hydrogen peroxide-induced damage to cellular DNA in human lymphocytes (Jeon *et al.*, 2006)

Figure 1-12 Glutinous purple rice *(Oryza sativa* L. var. *indica)*

Purple rice (*Oryza sativa* L. var. *indica*) also known as black rice or forbidden rice. The unique character is purple color of hull and pericarp. It presents high amount of flavonoids including anthocyanins especially cyanidin-3-glucoside, peonidin-3-glucoside and petunidin-3-glucoside (Yoshimura *et al.*, 2012). Anthocyanins rich fraction extract from Thai black sticky rice can be beneficial for health promotion by reducing oxidative stress and enhancing low-density lipoprotein (LDL) clearance, regulating LDL-receptor production on the cell surface membrane, thereby maintaining lipid homeostasis (Sangkitikomol *et al.*, 2010). Moreover, cyanidin-3-glucoside and peonidin-3-glucoside extracted from black rice presented cytoxicity against human breast cancer cells *in vitro* and *in vivo* by inducing apoptosis and suppressing angiogenesis (Hui *et al.*, 2010). The anticancer effects of anthocyanin-rich extract from black rice against human breast cancer cells in vitro and in vivo by inducing apoptosis and suppressing angiogenesis (Hui *et al.*, 2010).

Glutinous purple rice *(Oryza sativa* L. var. *indica)* variety Kum Doisaket is cultivated in northern Thailand. It contains high amount of flavonoids, including proanthocyanidin and anthocyanins that have several pharmacological and biological properties (Punyatong *et al.*, 2008). Kum Doisaket has the highest for contents of total phenolic compounds and cyanidin-3-glucoside as well as antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays, when compared to other varieties of rice,

including Riceberry, Kham, KDML 105 and Sinlek (Daiponmaka *et al.*, 2010). Kum Doisaket presents the highest γ- aminobutyric acid (GABA), a psychological stress-reducing agent, when compare to other purple rice including Kum Nan, Kum Phayao, Kum vietnam, Kum Doi Musur and Kum 88 082 (Karladee and Suriyong, 2012). GABA have an inhibitory action on leukemia cell proliferation and have a stimulatory action on the cancer cell apoptosis (Oh and Oh, 2004).

Furthermore, our group also studied genotoxicity and antigenotoxicity of various extracts of hull and seed of glutinous purple rice variety Kum Doisaket using Salmonella mutation assay. The n-hexane, dichloromethane, methanol, 95% ethanol and water of seed and hull extracts showed no mutagenicity in *Salmonella typhimurium* strains TA98 and TA100 in both the presence and absence of metabolic activation. These extracts inhibited the number of revertant colonies from aflatoxin B_1 and 2-amino-3, 4-dimethylimidazo[4,5-f]quinolone induced-mutagenesis. Among seed extracts, the methanol seed extract presented the strongest antimutagenicity. While the dichloromethane extract showed the most potent antimutagenicity compared to the other hull extracts. Using 0.1% HCl in methanol for seed and hull extraction obtained the highest amounts of total phenolic, total flavonoids and cyanidin-3-glucoside when compared to the other solvents. However, the acidified methanol extract of rice hull demonstrated both mutagenicity and antimutagenicity in a dose-dependent manner (Punvittayagul *et al.*, 2011).

1.3 Objectives of the study

1. To evaluate the toxicity of glutinous purple rice extracts in rats
2. To study the clastogenicity and anticlastogenicity of glutinous purple rice extracts using rat liver micronucleus test
3. To determine the effect of glutinous purple rice extracts on xenobiotic metabolizing enzymes