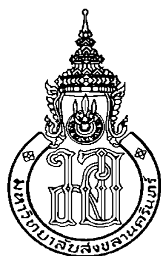

# Effect of Phenethyl Isothiocyanate (PEITC) and Betong watercress (*Nasturtium officinale*) on Glutathione *S*-transferase Activity and Glutathione Levels in Rats

**Baddariyah Sohsansa**

**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology**

**Prince of Songkla University**

**2014**

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**ชื่อวิทยานิพนธ์** ผลของเฟนเอทิลไอโซไธโอไซยาเนทและผักน้ำเบตงต่อแอคติวิตี้ของเอนไซม์กลูต้าไธโอนเอสทรานเฟอเรส และระดับของกลูต้าไธโอนในหนูขาว

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## บทคัดย่อ

เฟนเอทิลไอโซไทโอไซยาเนท (Phenethyl isothiocyanate ; PEITC) ซึ่งพบได้ในพืชตระกูลกะหล่ำปลี สามารถเหนี่ยวนำแอคติวิตี้ของเอนไซม์กลูต้าไธโอนเอสทรานเฟอเรส (GST) ได้ อย่างไรก็ตาม ผักน้ำเบตงเป็นพืชในตระกูลกะหล่ำปลี ที่พบมากในภาคใต้ของประเทศไทย ยังไม่มีรายงานวิจัยเกี่ยวกับผลต่อแอคติวิตี้ของเอนไซม์กลูต้าไธโอนเอสทรานเฟอเรสและระดับกลูต้าไธโอน การวิจัยนี้มีวัตถุประสงค์หลักเพื่อศึกษาผลของ PEITC และผักน้ำเบตงทั้งสดและปรุงสุกต่อแอคติวิตี้ของเอนไซม์กลูต้าไธโอนเอสทรานเฟอเรส และระดับของกลูต้าไธโอนในหนูขาวใหญ่

การทดลองใช้หนูขาวใหญ่สายพันธุ์ Wistar เพศผู้ น้ำหนัก 200 – 250 กรัม โดยแบ่งออกเป็น 11 กลุ่ม กลุ่มที่ 1 สัตว์ทดลองได้รับน้ำกลั่นปริมาณ 10 มิลลิลิตร/ กิโลกรัม กลุ่มที่ 2 ได้รับน้ำมันข้าวโพดปริมาณ 10 มิลลิลิตร/ กิโลกรัม กลุ่มที่ 3, 4 และ 5 ได้รับ PEITC ขนาด 25, 50 และ 100 มิลลิกรัม/กิโลกรัม กลุ่มที่ 6, 7 และ 8 ได้รับน้ำคั้นผักน้ำเบตงสด ขนาด 357, 714 และ 1428 มิลลิกรัมของผักสด/กิโลกรัม กลุ่มที่ 9, 10 และ 11 ได้รับน้ำคั้นผักน้ำเบตงปรุงสุก ขนาด 357, 714 และ 1428 มิลลิกรัม/กิโลกรัม ทางปาก วันละครั้งติดต่อกันเป็นเวลา 3 วัน และตรวจวัดแอคติวิตี้ของเอนไซม์ GST ในตับ ระดับของกลูตาไธโอนทั้งหมด รีดิวส์กลูตาไธโอน (GSH) ออกซิไดซ์กลูตาไธโอน (GSSG) รวมทั้งอัตราส่วนของรีดิวส์กลูตาไธโอนต่อออกซิไดซ์กลูตาไธโอน (GSH/ GSSG; redox ratio) จะถูกตรวจวัด โดยใช้เครื่องสเปกโตรโฟโตมิเตอร์

ผลการศึกษาพบว่าหนูกลุ่มที่ได้รับ PEITC ที่ขนาด 25, 50 และ 100 มิลลิกรัม/กิโลกรัม สามารถเพิ่มแอคติวิตี้ของเอนไซม์ GST ได้ถึง 16.9, 32.4 และ 51.4% ตามลำดับ แต่ไม่แตกต่างกันเมื่อเทียบกับกลุ่มที่ได้รับน้ำมันข้าวโพด หนูกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสดทุกขนาด สามารถเพิ่มแอคติวิตี้ของเอนไซม์ได้ถึง 57.4, 54.7 และ 93.4% ตามลำดับ แต่ไม่แตกต่างกันเมื่อเทียบกับกลุ่มที่ได้รับน้ำกลั่น หนูกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสุกทุกขนาดพบว่าสามารถเพิ่มแอคติวิตี้ของเอนไซม์ได้เช่นเดียวกัน ในหนูกลุ่มที่ได้รับน้ำคั้นผักน้ำสุกขนาด 714 มก/กก สามารถเพิ่มแอคติวิตี้ได้ถึง110.2% อย่างมีนัยสำคัญทางสถิติ ($p$<0.05) เมื่อเทียบ

กับกลุ่มที่ได้รับน้ำกลั่นและกลุ่มที่ได้รับผักน้ำเบตงสุกขนาด 1428 มก/กก และในกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสุกขนาด 1428 มก/กก สามารถเพิ่มแอคติวิตี้ได้ถึง 97.9% อย่างมีนัยสำคัญทางสถิติ ($p$<0.05) เมื่อเทียบกับกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสุกขนาด 357 มก/กก ในขณะเดียวกันยังพบว่าแอคตีวิตี้ของเอนไซม์ที่เพิ่มขึ้นมีผลสอดคล้องกับการเพิ่มขึ้นของปริมาณโปรตีนในตับ นอกจากนั้นยังพบว่าระดับของกลูตาไธโอนทั้งหมด และ GSH ลดลงหลังจากได้รับ PEITC แต่ไม่แตกต่างกับกลุ่มที่ได้รับน้ำมันข้าวโพด ส่วน GSSG มีค่าลดลงอย่างมีนัยสำคัญทางสถิติ ($p$<0.05) เมื่อเทียบกับกลุ่มที่ได้รับน้ำมันข้าวโพด ทำให้สัดส่วนของ redox ratio เพิ่มขึ้น ในหนูกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสดขนาด 1428 มก/กก พบว่าระดับของ GSH เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูกลุ่มที่ได้รับน้ำกลั่นและกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสดขนาด 714 มก/กก ($p$<0.05) ทำให้สัดส่วนของ redox ratio เพิ่มขึ้น และในหนูกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงปรุงสุกขนาด 357 มก/กก มีระดับของกลูตาไธโอนทั้งหมด และ GSH เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูกลุ่มที่ได้รับน้ำกลั่นและหนูกลุ่มที่ได้รับน้ำคั้นผักน้ำเบตงสดขนาด 714 มก/กก ($p$<0.05) และพบว่าสัดส่วนของ redox ratio ลดลงเมื่อได้รับน้ำคั้นจากผักน้ำเบตงปรุงสุกในขนาดเพิ่มขึ้น

โดยสรุปผักน้ำเบตงทั้งสดและปรุงสุกมีแนวโน้มในการเพิ่มแอคติวิตี้ของ GST ส่วนผลต่อกลูตาไธโอนของผักน้ำสดและปรุงสุกให้ผลที่แตกต่างกัน ดังนั้น ผักน้ำเบตงจึงเป็นที่น่าสนใจอย่างมากในการศึกษาการต้านพิษหรือใช้ในการป้องกันการเกิดโรคบางอย่างต่อไป

**Thesis Title** Effect of Phenethyl Isothiocyanate (PEITC) and Betong watercress (*Nasturtium officinale*) on Glutathione *S*-transferase Activity and Glutathione Levels in rats

**Author** Miss Baddariyah Sohsansa

**Major Program** Pharmacology

**Academic Year** 2013

## ABSTRACT

Phenethyl isothiocyanate (PEITC), which found in cruciferous vegetable, induces glutathione *S*-transferase (GST) activity. However, the effects of cruciferous vegetable namely Betong watercress, found in Southern part of Thailand, have not been demonstrated. The main objective of this study was to investigate the effects of PEITC, fresh and cooked Betong watercress on GST activity and glutathione levels in rats.

The experiments were carried out in male Wistar rats (200-250 g), which were divided into eleven groups. Group I served as control and the animals received deionized water at 10 mL/kgBW. In group II the animals were given corn oil at 10 mL/kgBW. In group III to V, rats were treated with PEITC at different doses of 25, 50 and 100 mg/kg. Animals in group VI to VIII were given fresh Betong watercress juice (FBWC) at dose of 357, 714 and 1428 mg/kgBW. And in group IX to XI, animals were given cooked Betong watercress juice (CBWC) at dose of 357, 714 and 1428 mg/kgBW. All rats were orally administered once a day for consecutive 3 days. The catalytic activity of liver GST was determined. The total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) were investigated by using spectrophotometric method. In addition, the calculating redox ratio (GSH/GSSG) was presented.

The results showed that the GST activities were not significantly increased after receiving the PEITC (25, 50, 100 mg/kg) when compared with corn oil treated group. The percentage increases of GST activity were 16.9, 32.4 and 51.4%, respectively, when compared with corn oil treated group. The percentage increases of

GST activity of FBWC were 57.4, 54.7 and 93.4% respectively, but not significantly difference when compared with deionized water treated group. In 714 mg/kgBW CBWC was significantly increased (110%) when compared with deionized water treated group and 714 mg/kgBW CBWC treated group ($p<0.05$). In 1428 mg/kgBW CBWC was significantly increased (97.9%) when compared with 357 mg/kgBW CBWC treated group. The increasing of GST activity was correlated with the increasing liver protein content. After administration of PEITC total glutathione, GSH and GSSG levels were not significantly decreased when compared with corn oil treated group. However, GSSG were significantly reduced when compared with corn oil treated group ($p< 0.05$). In PEITC treated groups redox ratio had tend to increase in regard of dose dependence. After administration of 1428 mg/kg FBWC GSH levels were significantly increased when compared with deionized water treated group and 714 mg/kgBW FBWC treated group ($p< 0.05$). In FBWC treated groups redox ratio gradually increased with dose dependence. After administration of 357 mg/kg CBWC total glutathione and GSH levels were significantly increased when compared with deionized water treated group and 714 mg/kg FBWC ($p< 0.05$). In contrast, the redox ratios of CBWC treated groups were not increase with dose

In conclusion, both fresh and cooked Betong watercress juice seemed to induce GST activity; however, the increase in redox ratio of CBWC treated group was not dose dependent. Therefore, Betong watercress would be interesting to investigated future for antidotal therapy and protective effect of some diseases.

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# List of Abbreviations

| | | |
|---|---|---|
| BCNU | = | 1, 3-bis(2-chloroethyl) -1-nitrosourea |
| BSA | = | bovine serum albumin |
| CDNB | = | 1-chloro-2,4-dinitrobenzene |
| CBWC | = | cooked Betong watercress |
| COMC | = | crotonyloxymethyl-2-cyclohexenone |
| CYP | = | cytochrome P450 |
| CuOOH | = | cumene hydroperoxide |
| DDT | = | dichlorodiphenyl trichloroethane |
| DTNB | = | 5,5-dithio-bis (2-nitrobenzoic acid) |
| ETC | = | electron transport chain |
| FBWC | = | fresh juice of Betong watercress |
| GCL | = | glutamate cysteine ligase |
| GR | = | glutathione reductase |
| GSH | = | reduced glutathione |
| GSPx | = | glutathione peroxidase |
| GSSG | = | oxidized glutathione |
| GST | = | glutathione *S*-transferase |
| $H_2O_2$ | = | hydrogen peroxide |
| HO• | = | hydroxyl radical |
| 5-HPETE | = | (*S*)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid |
| ITCs | = | isothiocyanate |
| IMM | = | inner mitochondrial membrane |
| MAPEG | = | membrane-associated proteins in eicosanoid and glutathione metabolism |
| MRP | = | multidrug resistance-associated protein |
| MnSOD | = | manganese superoxide dismutase |
| mM | = | milimolar |
| min | = | minute |
| µL | = | microlitre |

## List of Abbreviations (cont.)

| | | |
|---|---|---|
| µmole | = | micromole |
| NADPH | = | ß-Nicotinamide adenine dinucleotide 2'- phosphate reduced tetra sodium salt hydrate |
| NAPQI | = | N-acetyl-*p*-benzoquinone imine |
| NEM | = | N-ethylmaleimide |
| nm | = | nanometer |
| $O_2^-$ | = | superoxide anion |
| PBL | = | peripheral blood leukocytes |
| PEITC | = | phenethyl isothiocyanate |
| PhIP | = | 2-amino- 1- methyl-6-phenylimidazo[4,5-b]pyridine |
| QR | = | quinone reductase |
| ROS | = | reactive oxygen species |
| SE | = | standard error of mean |
| SSA | = | 5-Sulfosalicylic acid hydrate |
| TEA | = | triethanoamine |
| TNB | = | 5-thio-2-nitrobenzoic acid |
| TrxR | = | thioredoxin reductase |
| 2-VP | = | 2-vinylpyridine |
| BW | = | body weight |

# CHAPTER 1

# Introduction

## 1.1 Background and Rationale

Cellular detoxifications are important mechanism for maintaining health by providing protection against numerous noxious agents in the environment. The glutathione *S*-transferase enzyme (GST) is one of the most important enzymes that play a role in cellular defense from chemical reactive species. GSTs catalyze the conjugation reaction of reactive chemical with glutathione. Substrates of GST include alkyl or aryl halides, isothiocyanates, $\alpha,\beta$-unsaturated carbonyls, quinines and epoxide-containing chemicals. Hence, GST is often thought as a safety system within the cell. This system designs to remove any reactive metabolites produced during cellular processes. Reactive metabolites almost occur in phase I metabolism which can do any cellular damage through adduct formation with cellular macromolecules.

GST represents a major group of drug metabolizing enzymes in phase II of drug metabolism which provides a benefit on the cellular detoxification. The GST catalyzes nucleophilic attack by reduced glutathione (GSH) on nonpolar compounds that contain an electrophilic carbon, nitrogen, or sulphur atom (Wilce and Parker, 1994; Keen and Jakoby, 1978). The detoxification reaction produces water soluble product. Such compounds which have electrophilic centres chemicals include parent chemical such as 2-amino- 1- methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), benzo[n]pyrene, 7,12-dimethyl- benz[a]anthracene, 5-methylchrysene, and 4-nitroquinoline-N-oxide or metabolite of the carcinogens aflatoxin $B_1$. In addition, GST also detoxifies pesticides in both groups of organochlorine and organophosphate i.e alachlor, atrazine, lindane, and methyl parathion. The anticancer drugs 1,3-bis(2-chloroethyl) -1-nitrosourea (BCNU), chlorambucil, cyclophosphamide, melphalan, and thiotepa, as well as the antibiotic fosfomycin are also substrate of this enzyme. Moreover, GST also protects reactive oxygen species (ROS) such as the superoxide radical, hydrogen peroxide, and the hydroxyl radical (Hayes *et al.*, 2005). Evidence

suggests that the activity of this enzyme is a crucial factor for determining the sensitivity of cells in a broad spectrum of toxic chemicals. The induction of enzyme activity may change the detoxification profile of xenobiotic and reduces their toxicity which makes the harmful to living organism. Even though GST plays an important role in detoxifying xenobiotics, but GST also cause anticancer drug resistance. Like as many enzymes in drug metabolism, GST was induced by chemicals. A significant number of these chemical inducers occur naturally and, as they are found as non-nutrient components in vegetables and citrus fruits (Tsai *et al.*, 2011). One type of chemicals that reported as GST inducer is phenethyl isothiocyanate (PEITC) (Morse *et al.*, 1989).

PEITC, classified as isothiocyanate (ITCs), is a dietary naturally occurring compound in the *genus Brassica* and *Brassicaceae* family of cruciferous vegetables (Al-Shehbaz *et al.*, 2006, 2006) that are cauliflower, cabbage, brussels sprouts, kale, broccoli, and watercress and *genus Raphanus* is another type of plant that produces isothiocyanate (Stoner *et al.*, 1991). PEITC can inhibit Phases I enzymes, including the various type of cytochrome P450 enzymes. From the previous study in animal model, PEITC have cancer preventive activity against lung, esophagus (Stoner *et al.*, 1991) and breast cancer because it can inhibit enzyme in biotransformation of procarcinogen (Chung *et al.*, 1996; Nishikawa *et al.*, 1997,). PEITC has been reported in cell lines, these inhibited prostate cancer cell growth and induced apoptosis (Tang *et al.*, 2011). On the other hand, PEITC can also induce phases II enzymes, including GST and quinone reductases that inactivate carcinogens and promote their excretion. Induction of GST activity also prevents the toxic effect of reactive metabolite from many xenobiotics such as toxic metabolite of acetaminophen, N-acetyl-*p*-benzoquinone imine; NAPQI (Seo *et al.*, 2000) by catalyzed the conjugation of NAPQI with GSH.

Glutathione (γ-glutamyl-L-cysteinylglycine; GSH) is the most abundant low-molecular-weight thiol in living cell. GSH plays an important role in reduce number of free radical in the body. In oxidative stress status, it can be convert to oxidized glutathione (GSSG). The role of GSH has greatly advanced in biochemical and nutritional sciences. These studies have led to the free radical theory

of human diseases and to the advancement of nutritional therapies to improve GSH status under various pathological conditions (Townsend *et al*., 2003).

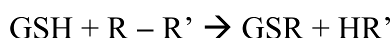
Watercress (*Nasturtium officinale*, R. Br.) is found growing naturally in abundance near springs, cool streams, or in moist soil on stream banks and open-running waterways in Europe, America and Asia. In addition, it is found in the South of Thailand, at Betong district, Yala province, which called "Betong watercress" in Thai. Currently, watercress is used as a medicinal plant (Bahramikia and Yazdanparast, 2010). This plant display a potential antibacterial activity, high hypolipidiaemic activity and antioxidant enzyme activity (Yazdanparast *et al.*, 2008). In addition, antimitotic and anticancer activities of this plant have also been reported (Potter and Steinmetz, 1996).

As watercress is a rich sources of glucosinolates nasturtiin, which is hydrolyzed by myrosinase resulting in PEITC production (Getahun and Chung, 1999). The effect of local watercress species in Thailand namely Betong watercress on GST activity or GSH level has not been studied yet. Therefore, the present study aimed to investigate the effects of multiple oral doses of PEITC and Betong watercress on GST activity and GSH level in rats. In this research, the effects of this vegetable were determined in two preparations: fresh and cooked Betong watercress.

## 1.2 Literature Review

### 1.2.1 Glutathione *S*-transferases (GST)

Glutathione transferases, GSTs, EC.2.5.1.18; known formerly as glutathione *S*-transferase that latter name gives rise to the used abbreviation, GST. GSTs are a polymorphic superfamily of multifunctional enzymes, which exist as homodimers and heterodimers . These enzymes found in evolutionary diverse species including bacteria, insects, plants, fish, birds, and mammals. These enzymes are contributed to the metabolism of drugs, pesticides, other xenobiotics and peroxide products from oxidative stress. These enzymes are plays a key role in phase II detoxification enzymes found mainly in the cytosol (Hayes *et al.*, 2005). The transferase possesses various activities and participates in several different types of reaction. Most of these enzymes can catalyse the conjugation of GSH with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulphur atom of GSH (Keen and Jakoby, 1978) and the substrate (R-R') (Armstrong, 1996). The general reactions showed (Armstrong, 1996):



$$GSH + R - R' \rightarrow GSR + HR'$$

In this role, GST action follows phase I of drug-metabolism which is often catalysed by members of the cytochrome P450 (CYP) supergene family. The CYP enzymes catalyze the introduction of a functional group, such as an epoxide, into an otherwise chemically inactive xenobiotic. This functional group offers an electrophilic centre that is attacked by GSH, the incoming nucleophile, in a reaction catalyzed by GST (Figure 1.1).

Like other phase I or phase II enzyme, GST can also be induced by a number of xenobiotics. Often, it is a compound that produces a reactive metabolite and which is then detoxified by GST that can induce the enzyme.

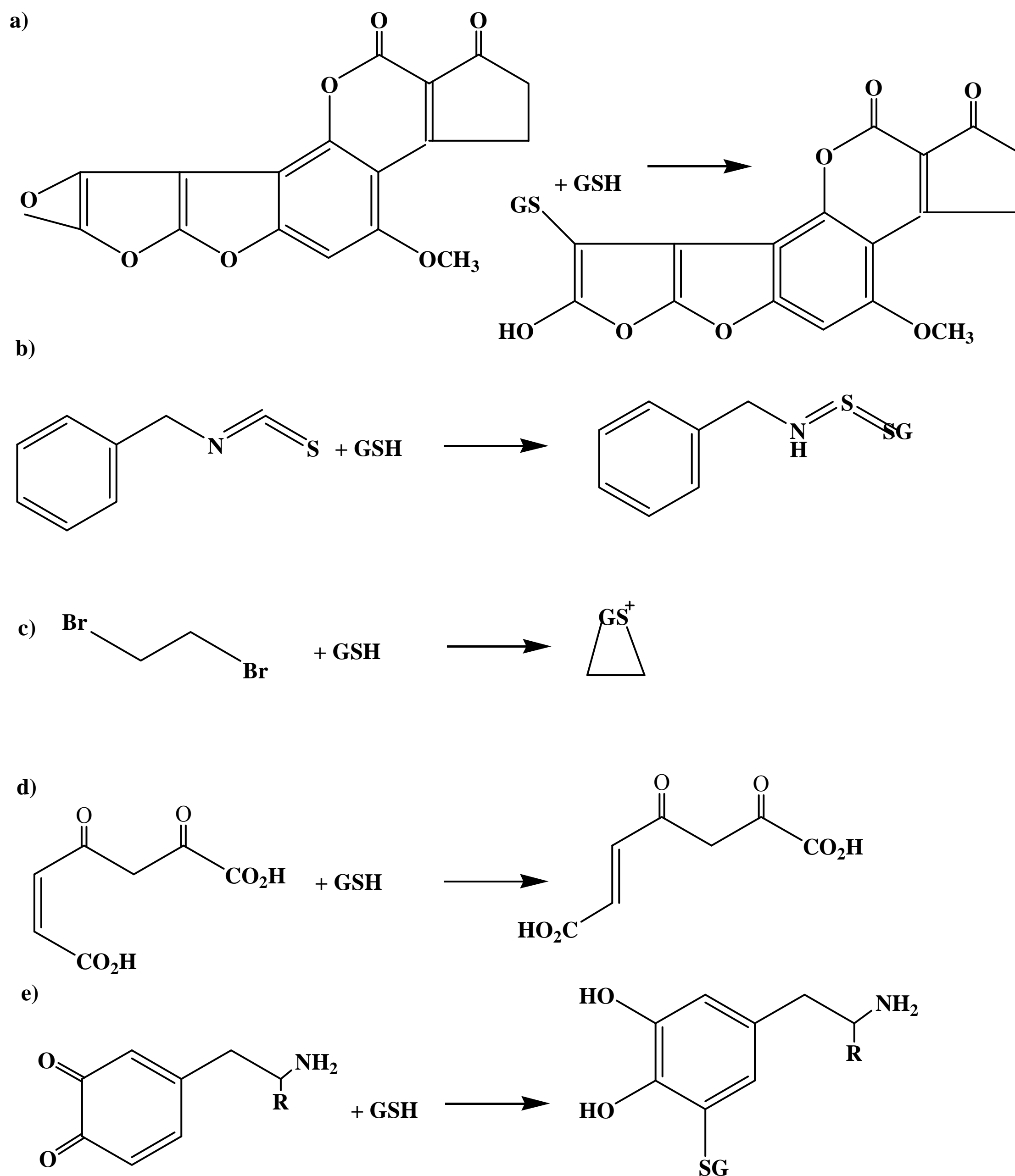



**Figure 1.1** Examples of GST catalysed reactions. The GST substrates shown are as follows: a, aflatoxin $B_1$-8,9-epoxide; b, benzylisothiocyanate; c, dibromoethane; d, maleylacetoacetate; e, a model *o*-quinone (Sherratt and Hayes, 2001).

#### 1.2.1.1 Classification of GSTs

Since the first GSTs were discovered as enzymes in 1961 (reviewed by Edalat, 2002), there has been an explosion in structural data across GSTs of three distinct superfamilies: the cytosolic, mitochondrial and microsomal proteins. The first superfamily enzyme was characterized cytosolic, or soluble, GSTs and involved in biotransformation of toxic xenobiotics and endobiotics. Soluble superfamily is composed of cytosolic and mitochondrial family. The other superfamily is the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG family) and involved in arachidonic acid metabolism. Mitochondrial GSTs and cytosolic, a dimeric enzyme, is a largest superfamily that contained at least 16 members in human. On the basis of their degree of sequence identity, the soluble mammalian enzymes have been assigned to eight families, or classes, designated as Greek letter are eight designated Alpha, Mu, Pi, Sigma, Theta, Omega, Zeta and Kappa (Table 1.1). Other classes of cytosolic GST namely Beta, Delta, Epsilon, Lambda, Phi, Tau, and the "U" class have been identified in non-mammalian species. Each class of GST cytosolic is present in various organs including liver, kidney, placenta, and red blood cell. The majority of cytosolic GST isoenzymes are found in the cytoplasm of the cell. Mitochondrial GST, referred to as GST Kappa. In mouse, rat, and human are a single Kappa GST class. In the mouse and rat, GST Kappa was presented in large amounts in liver, kidney, stomach, and heart (Thomson *et al.*, 2004). By contrast, GST Kappa appears to be more widely and uniformly expressed in human tissue (Morel *et al.*, 2004). And the MAPEG enzymes are four subgroups (I-IV) involved in the production of eicosanoids (Keen and Jakoby, 1978).

**Table 1.1** Summary of the Glutathione *S*-transferase (GST) classification.

| Superfamily | Family | Class |
|---|---|---|
| Soluble | Cytosolic | Alpha<br>Mu<br>Pi<br>Sigma<br>Theta<br>Zeta<br>Omega |
| | Mitochondrial | Kappa |
| MAPEG | Microsomal | I<br>II<br>III<br>IV |

#### 1.2.1.2 Tissue-specific regulation

The glutathione *S*-transferases are appeared in an organ-specific. In rat, class Alpha GSTs mainly are found in liver, kidney and small intestine. Class Mu GSTs are shown in liver, lung, heart, spleen, thymus, brain and testis. Class Pi GSTs was expressed in most extra hepatic tissue, class Sigma GSTs was presented in spleen, class Theta GSTs are found in liver, testis, adrenal gland, kidney and lung, and class kappa GSTs was appeared in liver. In the mouse, class Alpha, Mu, and Pi GSTs are shown in liver, lung, kidney, spleen, small intestine, heart, brain, testis and ovary. Class Theta GSTs are found in liver and lung. The Summary information about the tissue specific represent the tissue distribution of human soluble GSTs as shown in the table 1.2 The GSTs display overlapping substrate specificities, the substrates identified for each of the human GST are listed in table 1.2 (Sherratt and Hayes, 2001). From table 1.2, class Alpha, Mu, and Pi GST can detoxify $\alpha,\beta$-unsaturated carbonyls. Acroleine, 4-hydroxynonenal, aminochrome, dopachrome, noradrenochrome were also detoxified by these classes. Mu GST can detoxify aflatoxin $B_1$-8,9-epoxide. Class Pi enzymes

are active toward diol epoxides of benzo(a)pyrene, benzo(c)phenanthrene and benzo(g)chrysene.But cholesterol α-oxide is detoxified by class Alpha GST.

**Table1.2** Tissue distribution of human glutathione *S*-transferases

| Superfamily | Class | Protein | Organ | Substrates |
|---|---|---|---|---|
| Soluble | Alpha | GST1 | testis ≈ liver >> kidney ≈ adrenal > pancreas | CDNB; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; $\Delta^5$-androstene-3, 17-dione |
| | | GST2 | liver ≈ testis ≈ pancreas > kidney > adrenal > brain | CDNB; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; $\Delta^5$-androstene-3, 17-dione; CuOOH |
| | | GST3 | placenta | not determined |
| | | GST4 | small intestine ≈ spleen > liver ≈ kidney > brain | low with CDNB; Ethacrynic acid; 4-hydroxynonenal; 4-hydroxydecenal |
| Soluble | Mu | GSTM1 | liver > testis > brain > adrenal ≈ kidney > lung | CDNB; *trans*-4-phenyl-3-buten-2-one; aflatoxin $B_1$-epoxide; *trans*-stilbene oxide |
| | | GSTM2 | brain ≈ skeletal muscle ≈ testis > heart > kidney | high with CDNB; 1,2-dichloro-4-nitrobenzene; aminochrome |
| | | GSTM3 | testis >> brain ≈ small intestine > skeletal muscle | low towards CDNB; $H_2O_2$ |
| | | GSTM4 | testis | very low towards CDNB |
| | | GSTM5 | brain, heart, lung, testis | low towards CDNB |
| Soluble | Pi | GSTP1 | brain > heart ≈ lung ≈ testis > kidney ≈ pancreas | CDNB; acrolein; adenine propenal; BPDE; benzyl isothiocyanate; ethacrynic acid |
| Soluble | Sigma | GSTS1 | Fetal liver, bone marrow | prostaglandin $D_2$ synthase |
| Soluble | Theta | GSTT1 | kidney ≈ liver > small intestine > brain ≈ prostate | 1,2-epoxy-3-(p-nitrophenoxy)propane; dichloromethane; dibromoethane |
| | | GSTT2 | liver | CuOOH; 1-menaphthyl sulphate |
| Soluble | Zeta | GSTZ1 | fetal liver, skeletal muscle | dichloroacetate; fluoroacetate; malelyacetoacetate |
| Soluble | Omega | GSTO1 | liver ≈ heart ≈ skeletal muscle > pancreas > kidney | thioltransferase (very low with CDNB and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) |
| Soluble | Kappa | GSTK1 | liver (mitochondria) | not determined |
| MAPEG | (Microsomal) | MGST-I | liver ≈ pancreas > prostate > colon ≈ kidney > | CDNB; 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; 4-nitrobenzyl chloride; CuOOH |
| | | MGST-I- | brain | prostaglandin $D_2$ synthase |
| | | like I | testis > prostate > small intestine ≈ colon | CDNB; leukotriene $C_4$ synthase; 5-HPETE |
| | | MGST-II | liver ≈ skeletal muscle ≈ small intestine > testis | leukotriene $C_4$ synthase; 5-HPETE |
| MAPEG | (Microsomal) | MGST-III | heart > skeletal muscle ≈ adrenal gland, thyroid | leukotriene $C_4$ synthase |

| Superfamily | Class | Protein | Organ | Substrates |
|---|---|---|---|---|
| | | LTC$_4$S | platelets ≈ lung > liver | 5-lipoxygenase-activating protein (binds arachidonic acid) |
| | | FLAP | lung ≈ spleen ≈ thymus ≈ PBL >> small intestine | |

PBL: Peripheral blood leukocytes

CDNB: 1-chloro-2,4-dinitrobenzene

5-HPETE: (*S*)-5-hydroperoxy-8,11,14-*cis*-6-*trans*-eicosatetraenoic acid

CuOOH: Cumene hydroperoxide

**1.2.1.3 Role of GST in Metabolism**

**1.2.1.3.1 Metabolism of xenobiotics by GST**

**- Detoxification through the Mercapturic acid pathway.**

Glutathione *S*-transferases catalyze the first of four steps required for the synthesis of mercapturic acids (Figure 1.2). Subsequent reactions in this pathway entail sequential removal of *γ*-glutamyl moiety and glycine from the glutathione conjugate, followed finally by *N*-acetylation of the resulting cysteine conjugate. It is important to recognize that GST enzymes are part of an integrated defense strategy, and their effectiveness depends on the combined actions of, on one hand, glutamate cysteine ligase and glutathione synthase to supply GSH and, on the other hand, the actions of transporters to remove glutathione conjugates from the cell. Once formed, these conjugates are eliminated from the cell by the trans-membrane multidrug resistance-associated protein (MRP). Nine MRP proteins exist (Haimeur *et al*., 2004), and these are all members of the C family of ABC transporters. Among these, MRP1 and MRP2 can export glutathione conjugates and compounds complexed with GSH (Paumi *et al*., 2001). The dinitrophenol-glutathione ATPase called RLIP76 promotes efflux of glutathione conjugates from cell (Morrow *et al*., 2000), but as it is not a trans-membrane protein the mechanism is probably indirect.

Exogenous substrates for soluble GST include drugs, industrial intermediates, pesticides, herbicides, environment pollutants, and carcinogens. The cancer chemotherapeutic agents adriamycin, 1,3-bis(2-choroethyl)-1-nitrosourea (BCNU), busulfan, carmustine, chlorambucil, *cis*-plastin, crotonyloxymethyl-2-cyclohexenone (COMC-6), cyclophosphamide, ethacrynic acid, melphalan, mitozantrone, and thiotepa are detoxified by GST. Environmental chemicals and their metabolites detoxified by GST include acrolein, atrazine, dichlorodiphenyl trichloroethane (DDT), inorganic arsenic, lindane, malathion, methyl parathion, muconaldehyde, and tridiphane (Abel *et al*., 2004).

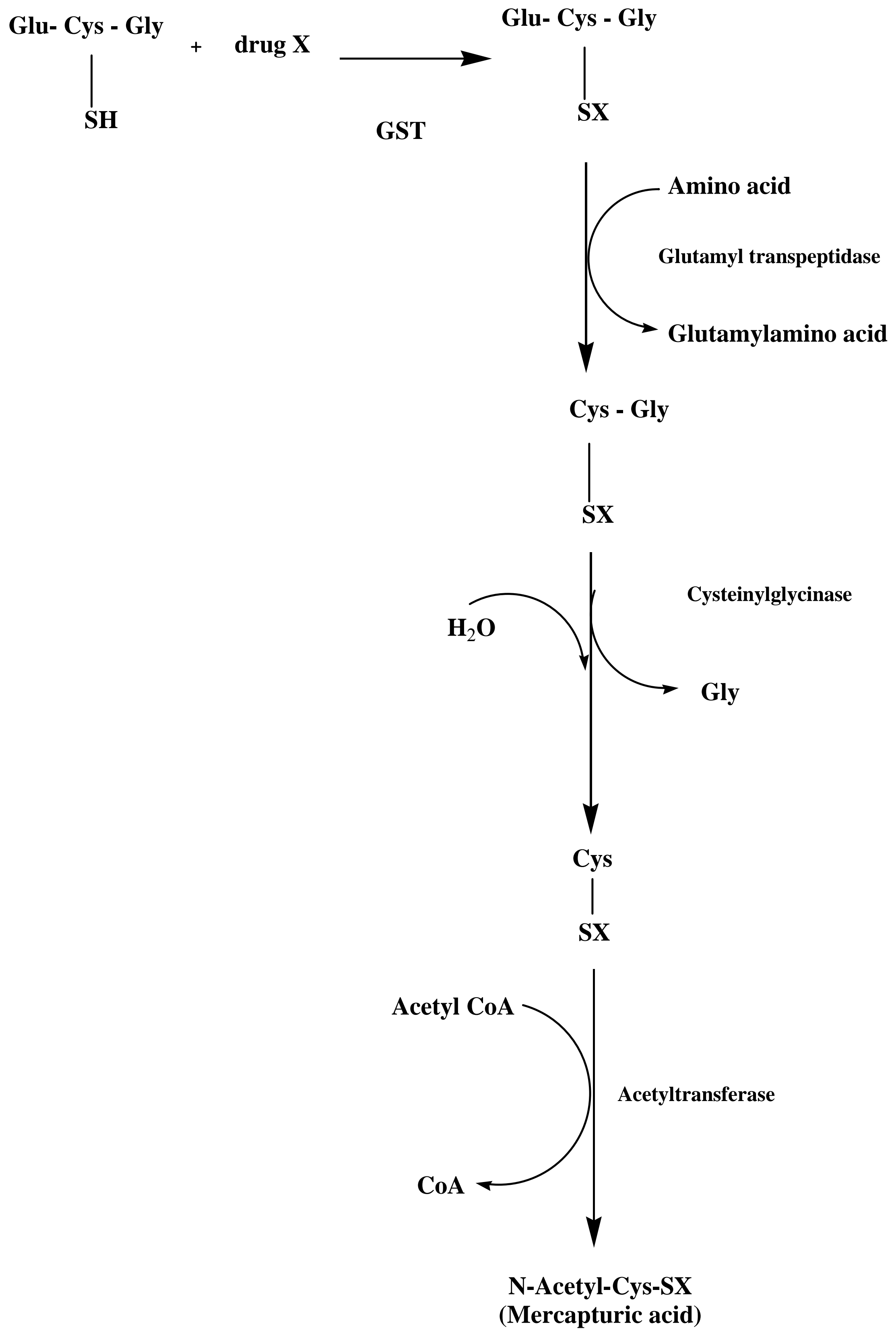



**Figure 1.2** The Mercapturic acid pathway

**- Bioactivation of xenobiotics by GST**

Conjugation of foreign compounds with GSH almost always leads to formation of less reactive products that are readily excreted. In a few instances, however, the glutathione conjugate is more reactive than the parent compound. Examples of this phenomenon are short-chain alkyl halides that contain two functional groups.

Catalyzing the conjugation of GSH with the solvent dichloromethane is resulting in the formation of the highly unstable *S*-chloromethylglutathione, which still contains an electrophilic center capable of modifying DNA (Wheeler *et al*., 2001). The 1,2-dihaloethanes are another group of GST substrates that are activated by conjugation with GSH to genotoxic products. However, in this instance, the glutathione conjugate rearranges to form an episulfonium intermediate that is responsible for modifying DNA (Guengerich *et al*., 2003).

Allyl-, benzyl-, phenethyl-isothiocyanates, and sulforaphane are moderately toxic compounds that are formed from plant glucosinolates. They are reversibly conjugated by GST with GSH to yield thiocarbamates spontaneously degrade to their isothiocyanates, liberating GSH. Thereafter, the isothiocyanate may be taken up again by the cell and reconjugated with GSH, only to be reexported as the thiocarbamate and revert to the isothicyanate. This cyclical process results in depletion of intracellular GSH and assists distribution of isothiocyanates throughout the body. Isothiocyanates should be taken up by cells that have a low GSH content, they may not be conjugated with GSH, but rather are more likely to thiocarbamylate proteins, a process that can result in cell death (Xu and Thornalley *et al*., 2001).

**1.2.1.3.2 Metabolism of endogenous compounds by GST**

**- Detoxification of products of oxidative stress**

The production of reactive oxygen species (ROS) such as superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (HO•), which is byproducts of incomplete oxygen metabolism. Mitochondria are unique organelles, as they are the main site of oxygen metabolism, accounting for approximately 85-90% of the oxygen consumed by the cell (Chance *et al*., 1979). It has been shown, however, that mitochondria produce ROS at a rate higher than their scavenging capacity,

resulting in the incomplete metabolism approximately 1-3% of the consumed oxygen. The formation of superoxide occurs via the transfer of a free electron to molecular oxygen. This reaction occurs at specific sites of the electron transport chain (ETC), which resides in the inner mitochondrial membrane. ETC complexes I (NADH dehydrogenase) and III (ubisemiquinone) (Boveris *et al.*, 1973) produce most of the superoxide, which is then scavenged by the mitochondria enzyme manganese superoxide dismutase (MnSOD) to produce $H_2O_2$ (Nohl and Hegner., 1977).

Ubiquinone(Ub), a component of the mitochondrial respiratory chain connecting Complex I with III and Complex II and III, is regarded as a major participant in formation of $O_2^-$ by Complex III. Mammalian complex I is the entry point for electrons from NADH into the respiratory chain. There is growing evidence that most of the $O_2^-$ generated by intact mammalian mitochondria in vitro is produced by Complex I; the contribution by other complexes and sites seem to be relatively low. This $O_2^-$ production occurs primarily on the matrix side of the inner mitochondrial membrane (IMM) (Brand *et al.*, 2004). $O_2^-$ production by Complex I was also found to be markedly stimulated in the presence of succinate, the substrate of complex II, indicating that a reverse electron flow is involved (Figure 1.3 A )(Orrenius *et al.*, 2007). The enzyme glutathione peroxidase (GSPx) requires reduced glutathione (GSH) as a coenzyme and converts $H_2O_2$ to water, thus completely detoxifying ROS. However, in the presence of reduced transition metals, $H_2O_2$ can produce the highly reactive OH•, which can cause extensive damage to DNA, proteins, and lipids (Nohl and Hegner, 1977; Orrenius *et al.*, 2007). Thioredoxin catalytic site contains two redox active cysteine which are involved in reversible reduction of protein disulfide bonds and are regenerated by Trx reductase (TrxR) and NADPH (Nakamura *et al.*, 1997). Among GSH-linked enzymes involved in mitochondrial antioxidant defense are glutathione peroxidases (Gpx) 1 and 4. Glutathione peroxidases catalyze the reduction of $H_2O_2$ and various hydroperoxidases, with GSH as the electron donor, Gpx1 is the major isoform and is localized predominantly in the cytosol, but a small fraction is also present within the mitochondrial matrix. In contrast, Gpx4 (also known as phospholipid hydroperoxide glutathione peroxidase) is membrane-associated, with a fraction localized to the intermembrane space of the mitochondria,

possibly at the contact sites of the two membranes (Figure 1.3 B) (review by Orrenius *et al.*, 2007).

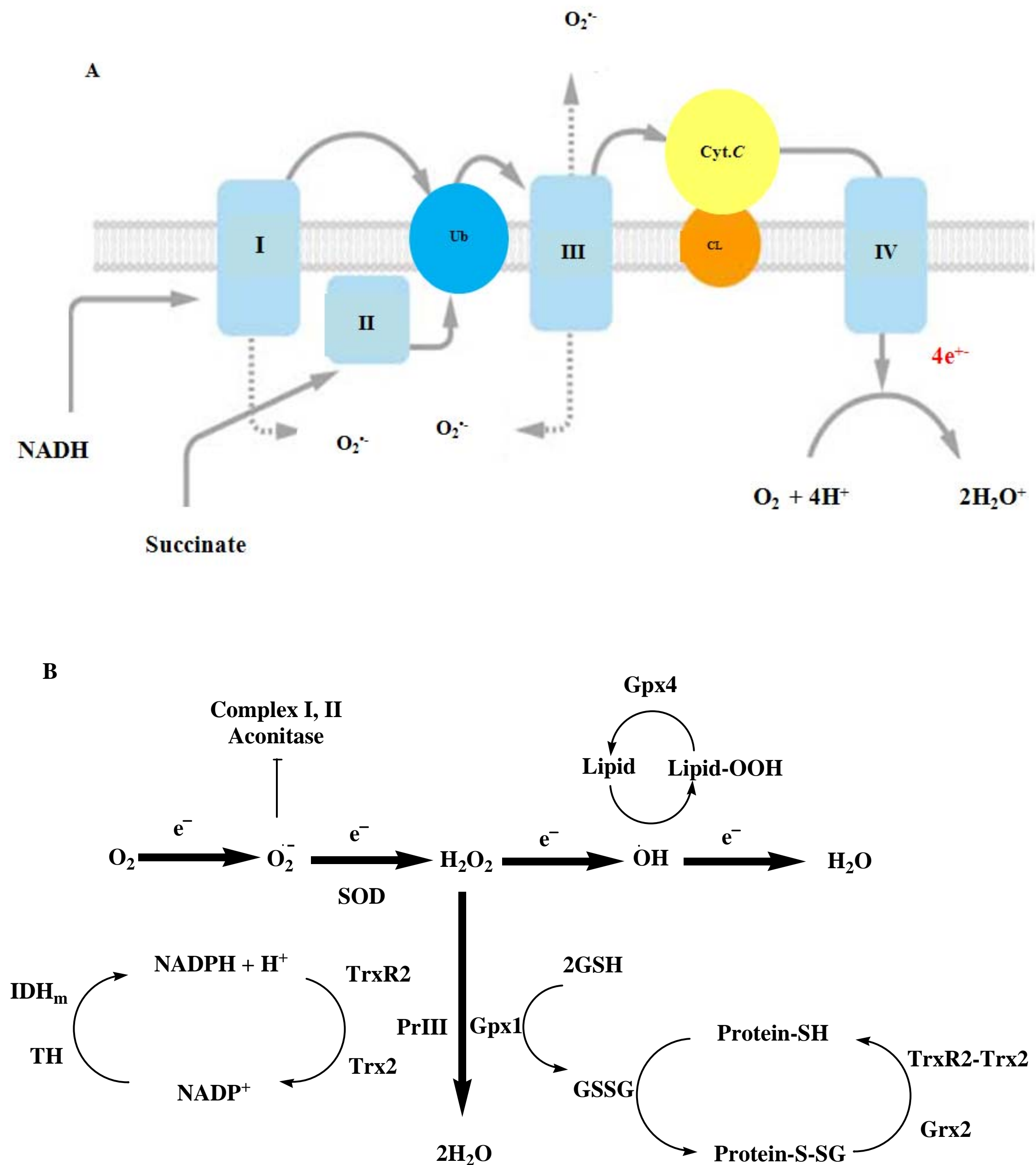



**Figure 1.3A**: Generators and targets of reactive oxygen species (ROS) in mitochondria**.** A schematic diagram of the generation of the main mitochondrial reactive oxygen species and their targets. **Figure 1.3B**: Formation, effects, and inactivation of reactive oxygen species in mitochondria (Orrenius *et al.*, 2007).

### 1.2.2 Phenethyl isothiocyanate (PEITC)

One type of several phytochemicals that extensively studied is phenethyl isothiocyanate (PEITC). It is also known as chemopreventive compound. PEITC is one of such important compounds from cruciferous vegetables.

#### 1.2.2.1 Source

PEITC is a dietary component classified into isothiocyanate (ITCs). ITC-containing compounds are characterized by sulfur containing N=C=S functional group and this compound is present in cruciferous vegetables. Vegetables of the Cruciferae family are in the botanical order Capparales, which includes the *Brassicas* genus. Examples are listed in table 1.3 (Wu *et al*., 2009). Crucifers belong to the family *Brassicaceae* and include *Brassica oleracea* (broccoli, cabbage, cauliflower, and brussels sprouts), *B. rapa* (Chinese cabbage and turnips), and several salad crops such as *Rorippa nasturtium-aquaticum* (watercress) and *Eruca sativa* (rocket) (Hu *et al*., 2003).

**Table 1.3** Vegetables and fruits of the family Cruciferae

| **Genus species** (sub species) | **Vegetable** |
|---|---|
| *Armoracia lapathifolia* | horseradish |
| *Brassica camoetris* (rapifera) | turnip |
| *Brassica camoestris* (oleifera) | rape |
| *Brassica napus* (napobrassica) | swede |
| *Brassica oleracea* (capitata) | white/red cabbage |
| *Brassica oleracea* (sabauda) | savoy cabbage |
| *Brassica oleracea* (gemmiifera) | brussel sprouts |
| *Brassica oleracea* (cauliflora) | cauliflower |
| *Brassica oleracea* (cymosa) | sprouting broccoli |
| *Brassica oleracea* (laciniata) | curly kale |
| *Brassica pekinensis* | chinese while cabbage |
| *Lepidium sativum* | garden cress |

| **Genus species** (sub species) | **Vegetable** |
|---|---|
| *Nasturtium officinale* | watercress |
| *Raphanus sativus* | radish |
| *Sinapis alba* | white mustard |
| *Carica papaya* | papaya |

**1.2.2.2 Chemical and physical properties**

The chemical structure is shown in figure 1.4

Synonyms: 2- phenylethyl isothiocyanate

2- isothiocyanatoethylbenzene

2-phenethyl isothiocyanate

1-(2-isothiocyanatoethyl) benzene

Formula: $C_6H_5CH_2CH_2NCS$.

Molecular weight: 163.24

Melting point: 75 °C

Density: 1.094 g/mL at 25 °C

Description: A light yellow liquid

Solubility: Insoluble in water

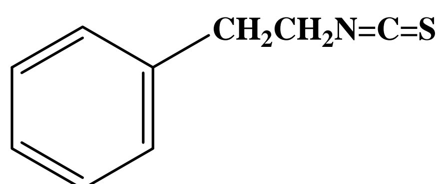



**Figure 1.4** Structure of PEITC (Ji *et al.*, 2005).

**1.2.2.3 Pharmacokinetic of PEITC**

PEITC are formed from glucosinolates, which are widely distributed among plants of the family *Brassicaceae*. The glucosinolates are not bioactive and appear to have no chemopreventive effects unless they are converted to ITCs. The general structures of glucosinolates consist of β-D-thioglucose group, a sulfonated

oxime group, and a side chain derived from methionine, phenylalanie, tryptophane, or branch-chained amino acids.

PEITC occur as glucosinolate precursors of hydrolysis by myrosinase as enzyme found in plants. Disruption of plant cells during harvesting, processing, or chewing releases myrosinase which comes into contact with glucosinolates and hydrolyzes them to different ITCs. PEITC is well absorbed and metabolized *in vivo* principally via the mercapturic acid pathway: an initial conjugation through the –N =C=S group with glutathione (GSH), which takes place spontaneously. But metanolism is further promoted by glutathione transferases (GST). The conjugates are then metabolized sequentially by γ-glutamyltranspeptidase, cystein glycinase, and N-acetyltransferase to finally form the mercapturic acids as shown in the figure 1.5 (Zhang, 2004). Excretion was rapidly within 24 h with peak plasma concentration in man on the range of micromolar.

Ji and colleaques (2005) reported male Sprague-Dawley rats were administered with PEITC at doses of 2, 10, 100, and 400 µmol/kg intravenously, or 10 and 100 mg/kg orally. PEITC had high oral bioavailability (115 and 93% at doses of 10 and 100 mmol/kg, respectively), low clearance (0.70 ± 0.17 L/h/kg at the lowest doses of 2 µmol/kg) and high plasma protein binding (86%). Nonlinear elimination and distribution were occurred following the administration of high doses.

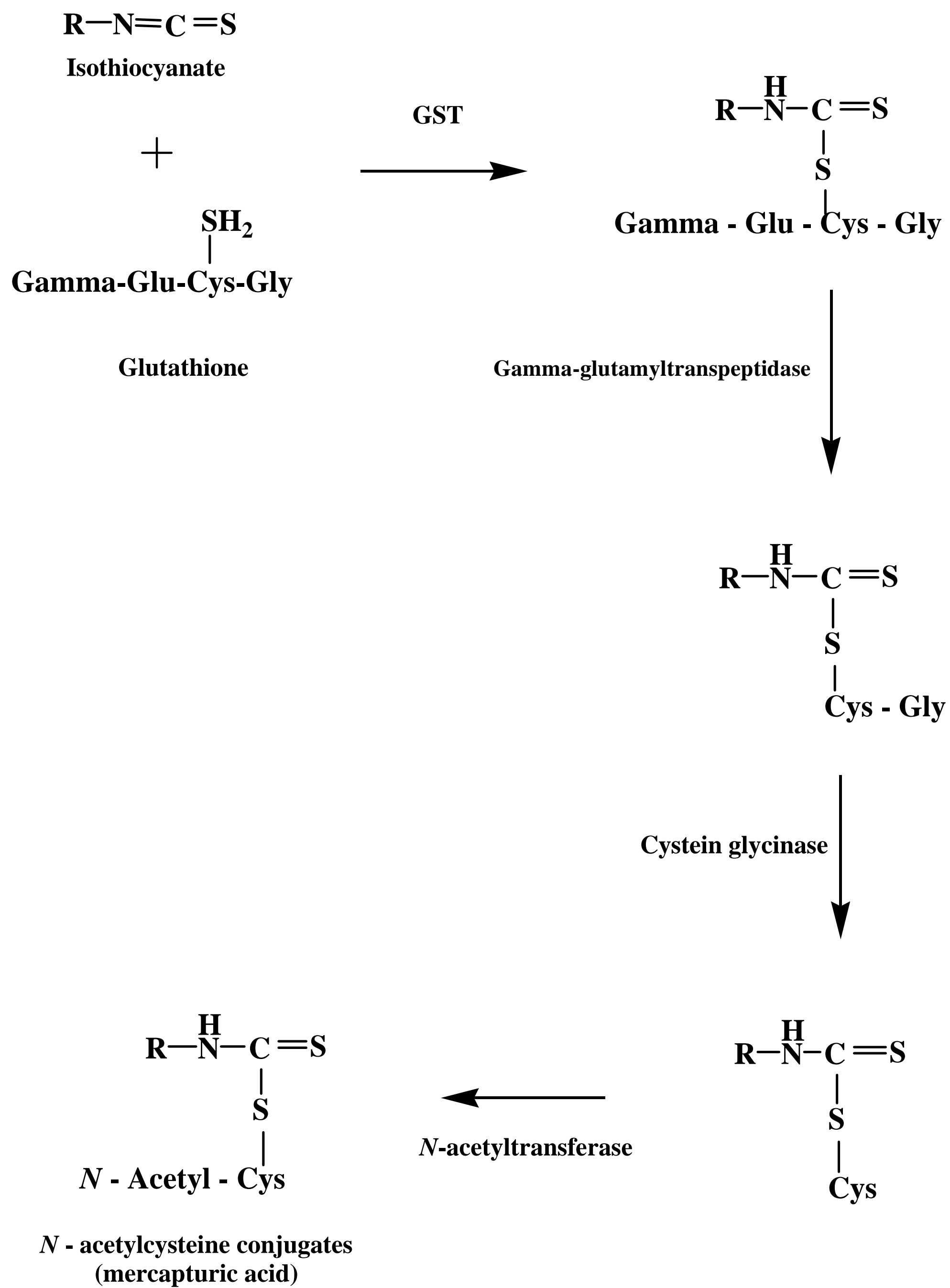



**Figure 1.5** The mercapturic acid pathway of isothiocyanate metabolism

**1.2.2.4 Pharmacological effect**

PEITC acts as both the metabolism involved in the inhibition of phase I enzymes such as cytochrome P450 (CYP 450) subtype 2E1 (CYP2E1) , subtype CYP1A2, subtype CYP3A and induction of phase II detoxification enzymes such as GST, NADPH quinine oxidoreductase and UDP-glucuronyl transferase (Meyer *et al.*, 1995).

**- Inhibition of phase I enzymes**

PEITC, a compound derived from cruciferous and other vegetable, is a potent inhibitor of cytochrome P450 2E1. This enzyme catalyzes the bioactivation of acetaminophen and many other xenobiotics. It has been reported that the effects of PEITC on acetaminophen metabolism associated with hepatotoxicity in Swiss-Webster mice. PEITC at dose of 19-150 µmole/kg was given to mice intragastrically 1 h before or immediately prior to a toxic dose of acetaminophen. The result shown that acetaminophen induced hepatotoxicity was significantly decreased or was completely prevented (Li *et al*.,1997). The protective action of PEITC against acetaminophen toxicity is attributed to the blocking of acetaminophen activation through inhibition of CYP450 enzymes. Morover, A/J mice were pretreated with PEITC for 4 consecutive days at daily doses of 5 or 25 $\mu$mole/kg inhibited tumor multiplicity induced by a single 10-µmole dose of NNK by approximatly 70% or 97%, respectively (Morse *et al*., 1989). Moreover Li and colleagues (1997) reported that in microsomal incubations, PEITC effectively inhibited the rate of acetaminophen-glutathione formation from acetaminophen as well as the CYP450 2E1 -dependent *N*-nitrosodimethlamine demethylase and the CYP450 (1A2)-dependent ethoxyresorufin *O*-deethylase activities.

Inaddition, rats were pretreated with sigle doses of PEITC at doses of 2-20 mg/kg decreases caffeine metabolic ratio (40-55%). PEITC 10 and 20 mg/kg were decreased significantly greater than those resulted from fluvoxamine. Caffeine metabolic ratios were also reduced after 5 day pretreatment with all doses of PEITC (43-69%). Reduction in metalic ratios caused by PEITC was doses independent both for single and multiple pretreatment. Such reduction was also time - independent. The findings indicated that PEITC inhibited CYP1A2 activity *in vivo* (Angkana, 2008).

**- Induction of phase II enzymes**

Isothiocyanate has shown to increase tissue levels of the phase II detoxification enzymes quinone reductas (QR) and glutathione *S*-transferase (GST) in a variety of rat tissues has been compared (Munday and Munday, 2004). In 2000, Seo and colleagues studied the induction of GST by PEITC in rats. It was found that 100 and 200 mg/kg orally administrated PEITC was increased GST activity 1.6 and 1.8 fold respectively. In addition, PEITC dosage ranging from 3.16 to 31.6 mg/kg were tended to increase GST activity but these changes were not statistically significant. PEITC has been shown to induce several phase II enzymes *in vivo*. When 1 mmol/kg (163.24 mg/kg) PEITC was orally administered to F344 rats orally, induction of NAD(P)H: quinine oxidoreductase and glutathione *S*-transferase activities were observed in the liver. In addition, sulfotransferase activity was induced in the nasal mucosa, while UDP glucuronosyl transferase activity was observed in the liver (Guo *et al*., 1992).

### 1.2.3 Betong watercress

Watercress (Figure 1.6) (*Nasturtium officinale*, R. Br.) is one of the cruciferous vegetables. It is in the family *Brassicaceae* or *Cruciferae*. Some commonly known plants in this family include broccoli, cabbage, cauliflower and brussels sprouts. *Brassicaceae* members share a suite of glucosinolate compounds, known as mustard oils, which are characteristic in identification of the family. The *Brassicaceae*, which alone contains more than 338 genera and 3,709 species. Of the many hundreds of cruciferous species are investigated to synthesize glucosinolates (Al-Shehbaz *et al.*, 2006).

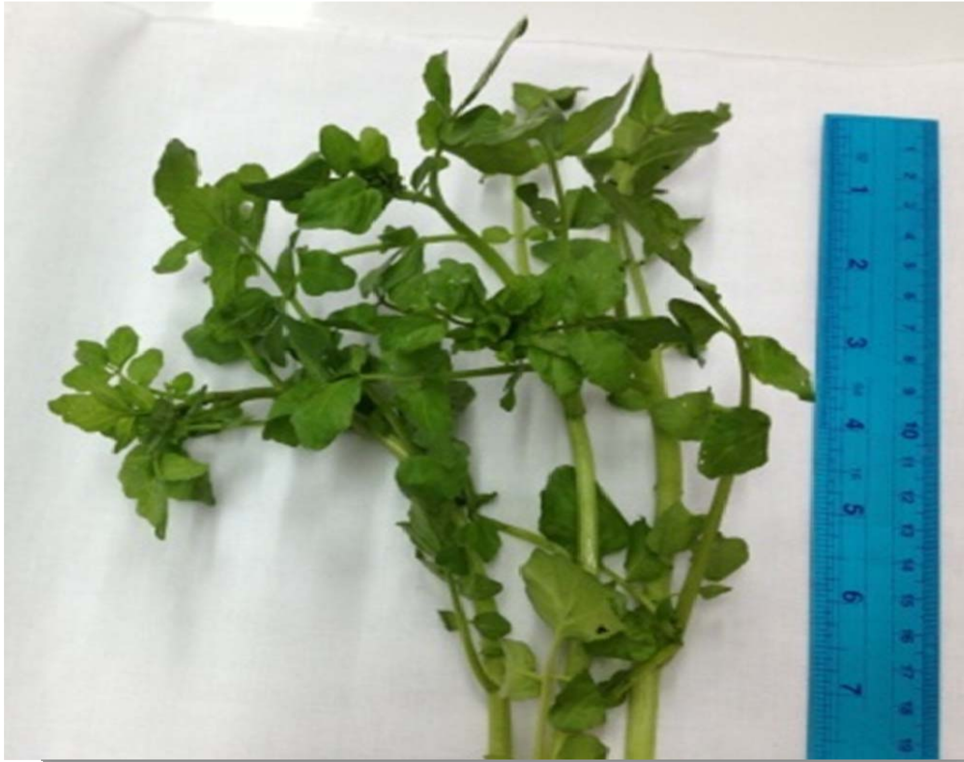


**Figure 1.6** Photograph of Betong watercress. (Photograped by Sathaporn Prutipanlai)

**1.2.3.1 Source**

Watercress is found growing naturally in abundance near springs, cool streams or in moist soil on stream banks and open-running waterways in Europe and Asia. In addition, it found in the Southern path of Thailand, at Betong, Yala province. Watercress growth is dependent on the nutrients necessary for its growth from the water. The most important nutrient is nitrogen (nitrate form). Moreover, high chloride content (greater than 1000 ppm) of the water or high pH (above 7.5) can also restrict watercress production. The optimum temperature for watercress growth are 21 to 29 $^{\circ}$C (Mchugh *et al*., 1987).

**1.2.3.2 Pharmacological effect**

The *Brassicaceae* , which contain several bioactive compounds with consequent antioxidant and other health-promoting properties. One of these vegetables is *Nasturtium officinale*, R. Br. or watercress used as a medicinal plant. Also, this plant have been reported that the leaves are traditionally used to treat depurative, diuretic, expectorant, hypoglycemic, odontalgic, stomach conditions, antimitotic and anticancer activities.

This plant is very nutritious with plant constituents including ascorbic acid (Vitamin C), calcium, folic acid, iron, iodine, and phosphorous. Moreover, it also contains arginine, glycine, lysine, tryptophan, antioxidant $\alpha$-tocopherol (Vit. E). Hadas reported that the iron level in watercress is more than in spinach, vitamin C is more than the orange and calcium level is more than the milk. One hundred gram fresh leaves of watercress is containing 43 mg vitamin C, 4700 unit vitamin A and 34 mg $\alpha$-tocopherol (reviwed by Jafari and Hassandokht, 2012).

In addition to nutrients, watercress is a rich source of a variety of phytochemicals. The most clearly identified include glucosinolate derivatives such as phenethyl isothiocyanate (PEITC) and methylsulphinylakyl isothiocyanates (MEITCs), flavonoids such as quercetin, hydroxycinnamic acids, and carotenoids such as beta-carotene (Vitamin A) and lutein (Lampe,1999).

Watercress is the richest source of glucosinolate nasturtiin, which are converted to isothiocyanates by the action of enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) (Figure 1.7). Glucosinolate nasturtiin is a kind of glucosinolate, one group of organic compounds, which include sulphur and nitrogen resulting from breaking glucose and one amino acid, these are the secondary metabolite in *Brassicaceae* family.

Isothiocyanate content of watercress were 7-metylsulphinylheptyl 0.1 μmol/g fresh weight (0.016 mg/g fresh weight), 8-metylsulphinylheptyl 0.06 μmol/g fresh weight (0.009 mg/g fresh weight), 3-indolylmethyl 0.04 μmol/g fresh weight (0.006 mg/g fresh weigh), 2-phenylethyl 1.53 μmol/g fresh weight (0.249 mg/g fresh weight), and 4-methoxy-3-indolylmethyl 0.065 μmol/g fresh weight (0.010 mg/g fresh weight) (Gill, 2007).

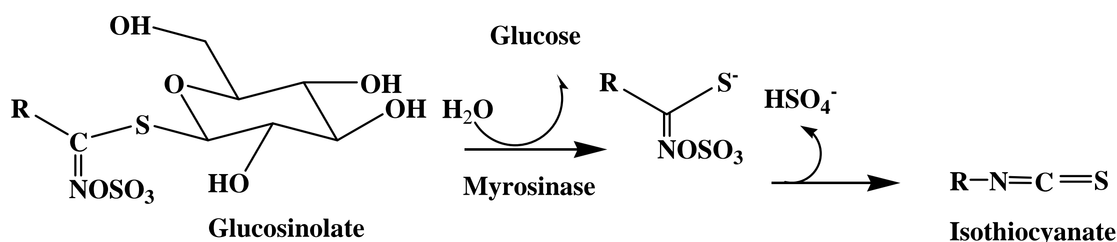
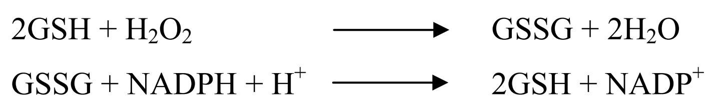



**Figure 1.7** The myrosinase-catalyzed conversion of glucosinolate to isothiocyanate (Zhang, 2004).

### 1.2.4 Glutathione

Glutathione (GSH) is a tripeptide thiol, γ-L-glutamyl-L-cysteinyl-glycine as shown in the figure 1.8 (Forman *et al.*, 2009). It is the major non-protein sulfhydryl compound in cells that is known to have numerous biological functions (Luberda, 2005). Cellular glutathione is normally present as the reduced form, only a small amount is usually less than 5% present as GSSG or mixed disulphides. Larger amounts of disulfides are found after a severe oxidative stress. In addition, intracellular glutathione is effectively maintained in the reduced state by a ubiquitous and crucial flavoenzyme, GSSG reductase (GR), and linked to the NADPH/NADH$^+$ system (Akerboom *et al.*,1981). The reactions are as follows:



$$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$$

$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

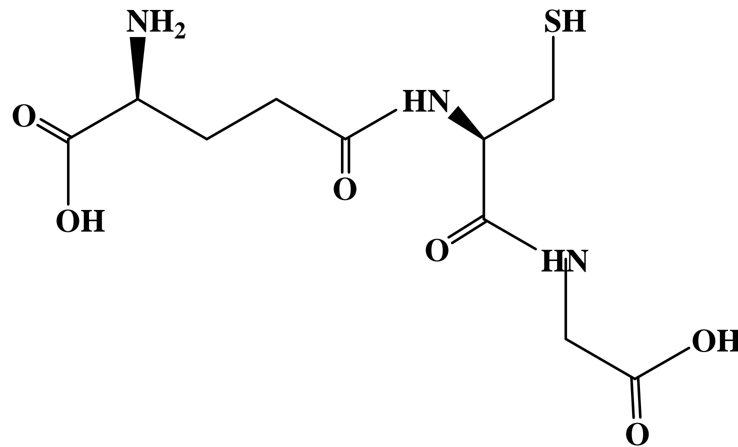



**Figure 1.8** Chemical structure of glutathione

**1.2.4.1 Glutathione Synthesis**

The synthesis of glutathione from its three amino acid precursors (glutamate, cysteine and glycine) takes place in the cytosol. Cell can make the necessary cysteine from the essential amino acid methionine. Glutathione is synthesized within the cell in two steps by the consecutive action of γ-glutamylcysteine synthetase and glutathione synthetase, as shown in the figure 1.9 (Luberda, 2005; reviewed by Forman, 2009).

The first step, cysteine and glutamate are combined by the enzyme glutamate cysteine ligase (GCL), which is also called γ-glutamylcysteine synthetase. The γ-glutamylcysteine synthetase are coupled ATP hydrolysis to form an amide bond between the γ-carboxyl group of glutamate and the amino group of cysteine. The second step, the addition of glycine to the γ-glutamylcysteine dipeptide, is catalyzed by glutathione synthetase to produce GSH (γ-glutamylcysteinylglycine) and also requires coupled ATP hydrolysis (reviewed by Forman, 2009).

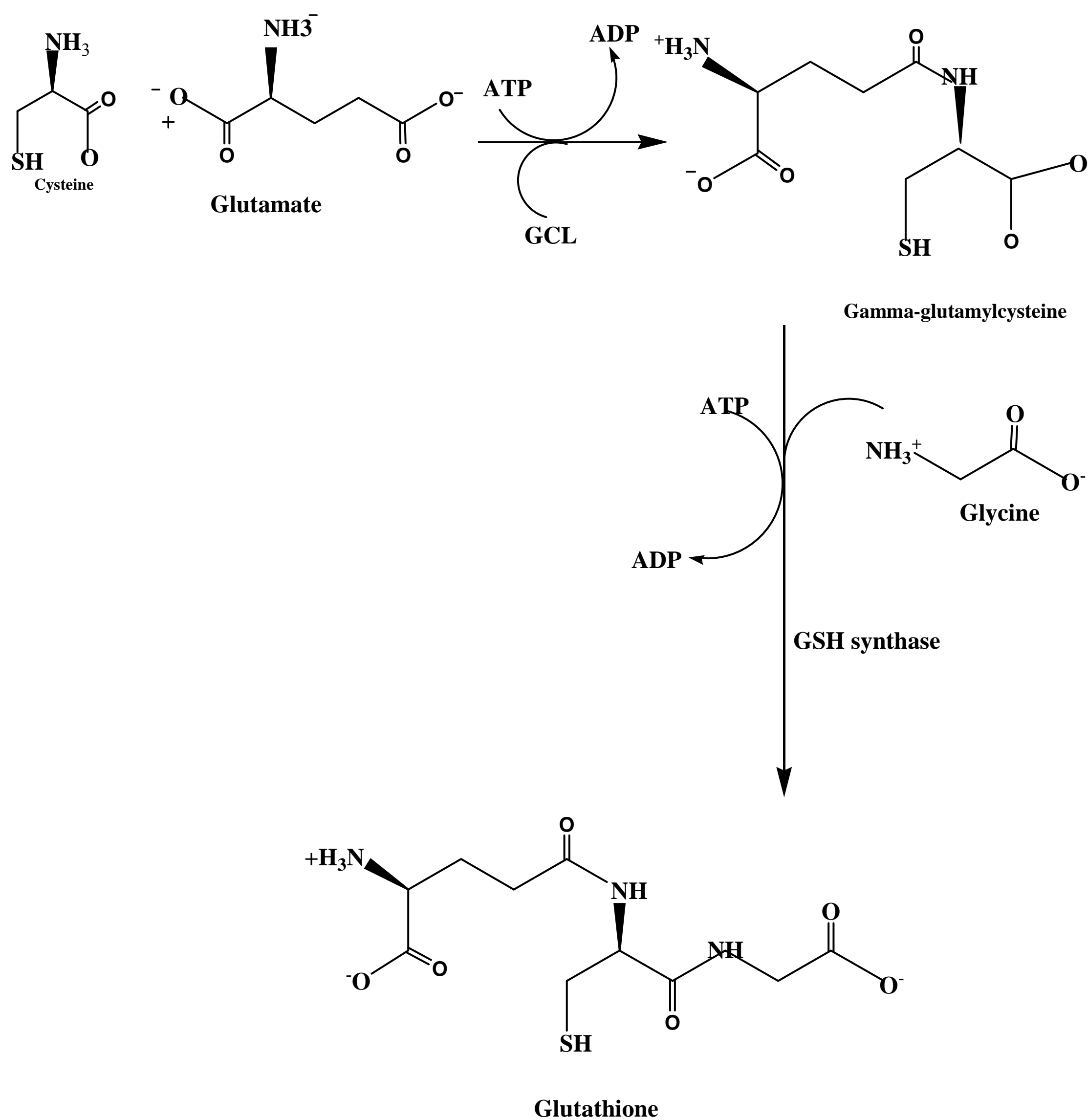



**Figure 1.9** Glutathione synthesis. The sequential ATP dependent formation of amide bond between cysteine and the γ-carboxyl group of glutamate and then glycine and cysteine are shown (Forman *et al.*, 2009). Abbreviation: GCL; glutamate cysteine ligase, ATP; adenosine triphosphate, ADP; adenosine diphosphate.

**1.2.4.2 Role of glutathione**

Glutathione participates in many cellular reactions. First, Glutathione effectively scavenges free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite, and $H_2O_2$) directly, and indirectly through enzymatic reactions (Fang *et al.*, 2002).

Second, glutathione reacts with various electrophiles, physiologicals metabolites (e.g., estrogen, melanins, prostaglandins, and leukotrienes), and xenobiotics (bromobenzene and acetaminophen) to form mercapturates. These reactions are initiated by glutathione *S*-transferase (Fang *et al*., 2002).

Third, GSH conjugates with NO to form an *S*-nitroso-glutathione adduct, which is cleaved by the thioredoxin system to release GSH and NO (Fang *et al*., 2002). Both, GSH and NO are necessary for the hepatic action of insulin-sensitizing agents, indicating their critical role in regulating lipid, glucose, and amino acid utilization (Guarino *et al.*, 2003).

Fourth, GSH serves as a substrate for formaldehyde dehydrogenase, whice converts formaldehyde and GSH to S-formyl-glutathione (Townsend *et al.*, 2003). The removal of formaldehyde (a carcinogen) is of physiological importance, because it is produced from the metabolism of methionine, choline, methanol (alcohol dehydrogenase), sacosine (sacosine oxidase), and xenobiotics (via the cytochrome P450-dependent monoxygenase system of the endoplasmic reticulum).

Fifth, GSH is required for the conversion of prostaglandin $H_2$ (a metabolite of arachidonic acids) in to prostaglandins $D_2$ and $E_2$ by endoperoxide isomerase (Lu, 2009).

Sixth, GSH is involved in the glyoxalase system, which converts methylglyoxal to D-lactate, an active pathway in microorganisms. Finally, glutathionylation of proteins (e.g., thioredoxin, ubiquitin-conjugating enzyme, and cytochrome c oxidase) plays an important role in cell physiology (Townsend *et al*., 2003).

Thus, GSH serves vital functions in animals (Table 1.4) adequate GSH concentrations are necessary for the proliferation of cells, including lymphocytes and intestinal epithelial cells (Aw, 2003). Glutathione also plays an important role in spermatogenesis and sperm maturation (Sies, 1999). In addition, GSH is essential for

the activation of T-lymphocytes and polymorphonuclear leukocytes as well as for cytokine production, and therefore for mounting succesful immune responese when the host is immunologically challenged (Townsend *et al*., 2003).

**Table 1.4** Roles of glutathione in animals

| |
|---|
| **Antioxidant defense** |
| Scavenging free radicals and other reactive species |
| Removing hydrogen and lipid peroxides |
| Preventing oxidation of biomolecules |
| **Metabolism** |
| Synthesis of leukotrienes and prostaglandins |
| Conversion of formaldehyde to formate |
| Production of D-lactate from methylglyoxal |
| Formation of mercapturates from electrophiles |
| Formation of glutathione-NO adduct |
| Storage and transport of cysteine |
| **Regulation** |
| Intracellular redox status |
| Signal transduction and gene expression |
| DNA and protein synthesis, and proteolysis |
| Cell proliferation and apoptosis |
| Cytokine production and immune response |
| Protein glutathionylation |
| Mitochondrial function and integrity |

Glutathione also regulates protein and DNA synthesis and participates in microtubule assembly (Luciano *et al.*, 2006). When mammalian cells are exposed to oxidative stress, the ratio of GSH/GSSG decreases due to the accumulation of GSSG. On this basis, the redued glutathione/oxidized glutathione ratio (GSH/GSSG ratio) is used to evaluate oxidative stress status in biological system. Oxidative stress has been recognized in different chronic diseases and has been assessed by blood GSSG levels (Lang *et al.*, 2000). A decrease in GSH concentrations has been implicated in several pathological conditions, such as diabetes, alcoholic liver disease, acquired immunodeficiency syndrome (AIDS), cataract, rheumatoid arthitis, muscular dysfunction, amyotrophic lateral sclerosis, Alzheimer's disease, respiratory distress syndrome, acute hemorrhagic gastric erosions, xenobiotic-induced oxidative stress (Pastore *et al.*, 2003).

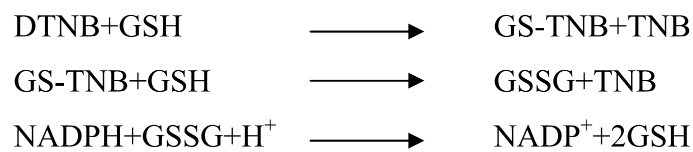
#### 1.2.4.2 Measurement of glutathione

Most of currently available methods that can measure both GSH and GSSG are based on either high-performance liquid chromatography or measurement of total glutathione by an enzymatic recycling method before and after masking of reduced glutathione using a thiol reagent (Tietze, 1969; Griffith, 1980; Baker *et al.*, 1990).

The enzymatic recycling methods for measurement of total glutathione and GSSG levels in biological samples are based on the reaction of GSH with the thiol-masking reagents N-ethylmaleimide (NEM) or 2-vinylpyridine (2-VP). Tietze (1969) used NEM to form a stable complex with GSH, preventing it from participating in the enzymatic recycling reaction with glutathione reductase (GR). Although this reaction is very effective (100% removal of GSH), it is relatively slow (40-60 min). Furthermore, the excess NEM has to be removed before the recycling reaction because it inhibits GR (Tietze, 1969). Later Giffith (1980) reported the use of 2-VP instead of NEM. 2-VP does not inhibit GR and therefore need not be removed from the sample. However, the masking reaction still requires 20-60 min incubation at room temperature. Because glutathione is unstable and may undergo autooxidation to GSSG at room temperature in some biological samples, a more rapid masking reaction is desirable (Griffith, 1980). Enzymatic assays are advantageous as high

specificity is guaranteed, contrary to orther methods that involve a non-specific derivatization of the GSH sulfhydryl group.

The glutathione determination is based on an enzymatic recycling assay of the 5,5-dithio-bis (2-nitrobenzoic acid)-glutathione disulfide reductase (DTNB-GSSG reductase). The DTNB-GSSG reductase recycling assay is a specific and sensitive procedure, as indicated in reaction (shown in the equations) (Anderson, 1985).



$$\text{DTNB+GSH} \longrightarrow \text{GS-TNB+TNB}$$
$$\text{GS-TNB+GSH} \longrightarrow \text{GSSG+TNB}$$
$$\text{NADPH+GSSG+H}^{+} \longrightarrow \text{NADP}^{+}\text{+2GSH}$$

The reduced form of glutathione (GSH) is oxidized by 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) to give glutathione disulfide (GSSG) with formation of 5-thio-2-nitrobenzoic acid (TNB). GSSG is reduced to GSH by the action of the highly specific DTNB-GSSG reductase and NADPH. The rate of TNB formation is followed at 412 nm and is proportional to GSH (Figure 1.10) (Forman *et al*., 2009).

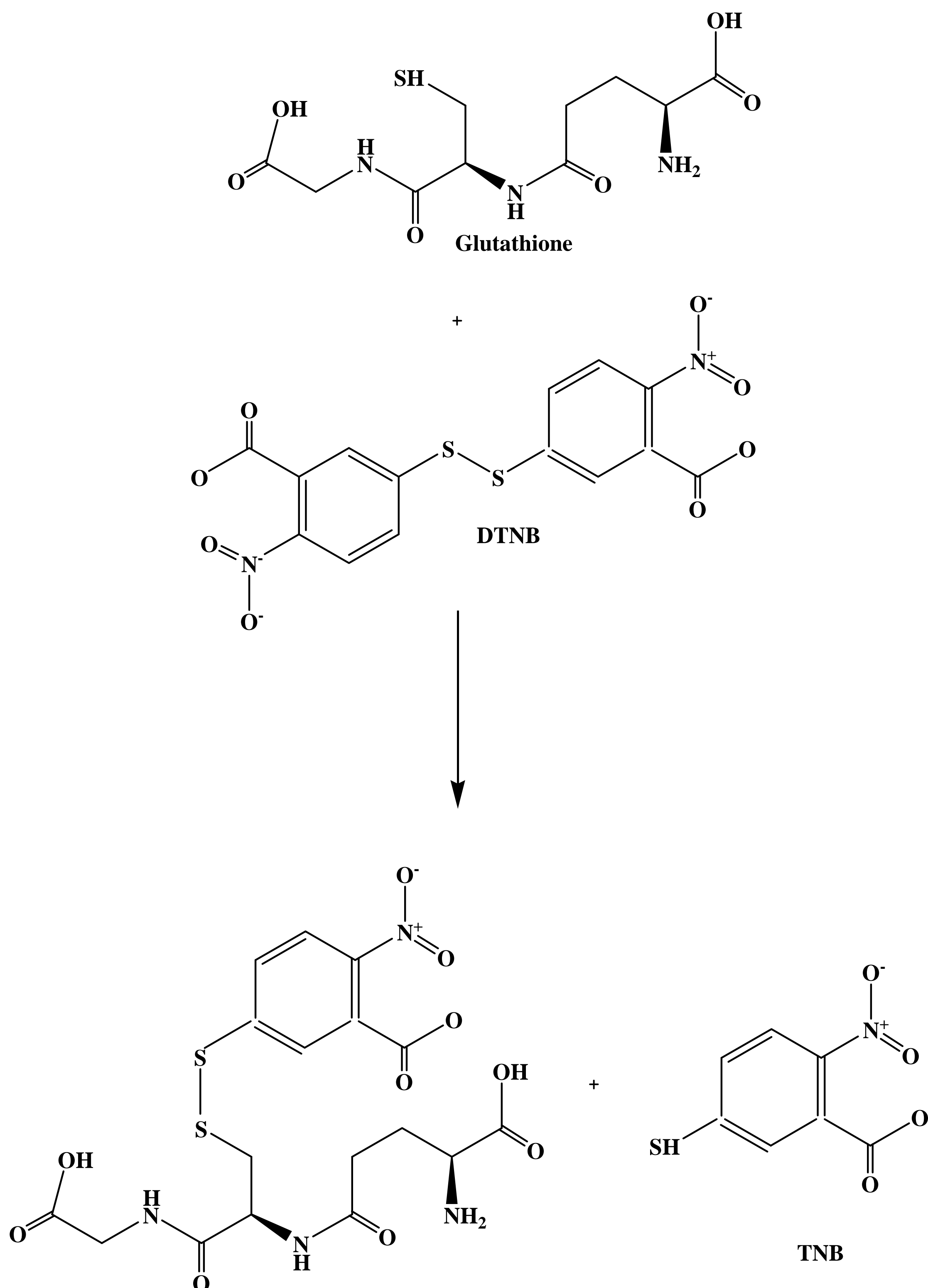



**Figure 1.10** The DTNB-GSSG reductase recycling system (Forman *et al.*, 2009).

## 1.3 Objectives

The objectives of this work were to study the influence of PEITC and Betong watercress on glutathione level and GST activity. The effects of different multiple doses of PEITC and Betong watercress were investigated. In addition, the effects of different doses of fresh and cooked Betong watercress juice on GSH and GST were investigated. The oxidative status in the liver after orally administrated multiple doses of PEITC, fresh and cooked Betong watercress juice was also investigated in rats.

# CHAPTER 2

# Methodology

## 2.1 Chemicals and Instruments

### 2.1.1 Chemicals

Chemicals were listed in Table 2.1.

**Table 2.1** A list of chemicals.

| Chemical | Chemical structure | MW (g/mole) | Supplier |
|---|---|---|---|
| Phenethyl isothiocyanate (PEITC) | $C_7H_8N_4O_2$ | 163.24 | Sigma-Aldrich, St. Louis, MO, USA |
| ß-Nicotinamide adenine dinucleotide 2'- phosphate reduced tetra sodium salt hydrate (NADPH) | $C_{21}H_{26}N_7Na_4O_{17}P_3$ | 833.35 | Sigma-Aldrich, St. Louis, MO, USA |
| Thiopental sodium | $C_{11}H_{17}N_2NaO_2S$ | 264.32 | Jagsonpal Pharmaceuticals Ltd, Haryana, India |
| 5-Sulfosalicylic acid hydrate (SSA) | $C_7H_6O_6S$ | 218.18 | Sigma-Aldrich, St. Louis, MO, USA |
| L-Glutathione reduced (GSH) | $H_2NCH(CO_2H)CH_2CH_2CONHCH(CH_2SH)CONHCH_2CO_2H$ | 307.32 | Sigma-Aldrich, St. Louis, MO, USA |
| Glutathione reductase from baker's yeast (GR) | | - | Sigma-Aldrich, St. Louis, MO, USA |
| Glutathione oxidized (GSSG) | $C_{20}H_{32}N_6O_{12}S_2$ | 612.63 | Sigma-Aldrich, St. Louis, MO, USA |

| Chemical | Chemical structure | MW (g/mole) | Supplier |
|---|---|---|---|
| 5,5'- Dithio-bis (2-nitrobenzoic acid) (DTNB) | $C_{14}H_8N_2O_8S_2$ | 396.3 | Sigma-Aldrich, St. Louis, MO, USA |
| Dibasic sodium phosphate | $Na_2HPO_4.2H_2O$ | 177.99 | Loba Chemie, Colaba, Mumbai, India |
| Monobasic sodium phosphate | $NaH_2PO_4.2H_2O$ | 156.01 | Fisher, Loughborough, Leicestershire, UK |
| Potassium dihydrogen phosphate | $KH_2PO_4$ | 136.09 | Ajex Finechem, Taren Point, NSW, Australia |
| Dipotassium hydrogen phosphate | $K_2HPO_4$ | 174.18 | Ajex Finechem, Taren Point, NSW, Australia |
| Triethanoamine (TEA) | $(C_2H_5)3N$ | 101.19 | Ajex Finechem, Taren Point, NSW, Australia |
| Sodium Chloride | NaCl | 58.44 | VWR International Ltd, Poole, Dorset , UK |
| 1-Chloro-2,4-dinitrobenzene (CDNB) | $(ClC_6H_3(NO_2)_2)$ | 202.55 | Merck, Darmstadt, Frankfurter, Germany |
| Ethylenediaminetetraacetic acid tetrasodium salt hydrate, | $(C_{10}H_{12}N_2Na_4O_8)$ | 380.17 | Sigma-Aldrich, St. Louis, MO, USA |
| Bio-Rad protein assay dry reagent | | - | USABio-Rad, Philadelphia, USA |
| Bovine serum albumin (BSA) | | - | Fluka-biochemika, Switzerland |

### 2.1.2 Instruments

Lists of instruments were shown in Table 2.2.

**Table 2.2** A list of instruments.

| Instrument | Source |
|---|---|
| Automatic pipette 1, 200 and 1000 μL | Gilson, Middleton, USA |
| Automatic pipette 5 mL | Brand, Wertheim, Germany |
| pH Meter | Mettler Toledo, Columbus, Ohio, USA |
| Ika® T-10 Basic Ultra Turrax Homogenizer | Optics Planet Inc, Illinois, USA |
| Sonics Vibra Cell® | Sonics & Materials, Newtown, USA |
| Spectronic Genesys20® | Thermo Scientific, Waltham, USA |
| Vortex Genie-2 | Scientific Industries, New York, USA |
| Water bath | Thermo Scientific, Waltham, USA |

### 2.2 Animals

Six-weeks-old male Wistar rats weighing between 200 to 250 g were obtained from Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai campus. Experimental protocol was approved by the Animal Ethic Committee, Prince of Songkla University (Ref. 12/55). Rats were housed 1 animal per cage. It were kept in environmentally controlled room with temperature 23 ± 2$^{o}$C, humidity 55% and 12 hour light/dark cycle and had free access to regular pellet diet and tap water *ad libitum*. The rats were fasted overnight but had free access to water prior to treatment.

### 2.3 Experimental design

Rats were randomly divided into 11 groups as followed (Figure 2.1). Rats in group I served as control are administered with deionized water at 10 mL/kg. In group II they were received corn oil at 10 mL/kg. In group III to V, rats were treated with PEITC (dissolved in corn oil) at different doses of 25, 50 and 100 mg/kg. Rats in group VI to VIII were received fresh Betong watercress (FBWC) at 357, 714 and 1428 mg /kg BW. Rats in group IX to X were received cooked Betong watercress (CBWC) at 357, 714 and 1428 mg /kg BW. Dose used for Betong watercress treatment was calculated following the recommended dose in adult human (50 g of fresh watercress/70 kg BW). The rats were treated as explained once a day for 3 consecutive days and at day 4$^{th}$ of the experiment the animals were anesthetized by thiopental sodium in order to collect the livers.

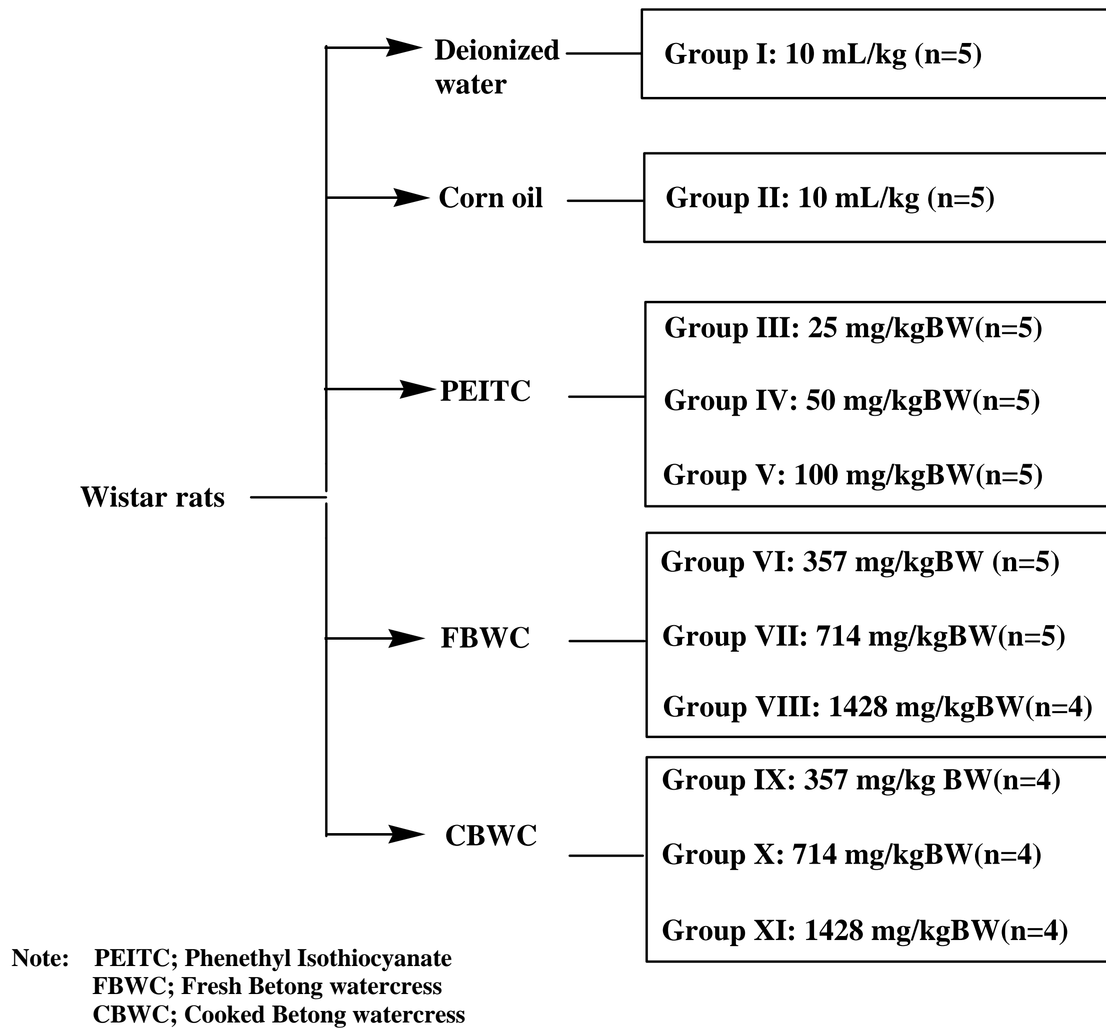



**Figure 2.1** The schematic diagram of experimental design

## 2.4 Methods

### 2.4.1 Liver sample collection

Rats were anesthetized with thiopental sodium. The abdomen of rat was cut with scissors to expose the thorax. A thoracectomy was performed and heart was rapidly perfused with 0.9 % normal saline. At the end of the perfusion, connective tissue of the liver was quickly removed. The liver was weighed, wrapped in foil and immediately freezed in liquid nitrogen and rapidly stored at -80°C until assayed.

### 2.4.2 Liver sample preparation

Liver tissue was homogenized in double distilled water for GST, 5 mL of 5% sulfosalicylic acid (SSA) for GSH and GSSG determine by using a tissue homogenizer (Ika® T-10 Basic Ultra Turrax Homogenizer) and ultrasonic tissue processor (Sonics Vibra Cell®) in an ice-chilled tube. The homogenate was centrifuged at 10,000 × g for 1.5 hours for GST, at 10,000 × g for 10 minutes for GSH and GSSG determine by centrifuge (Beckman coulter®). Supernatant was collected and stored in aliquots at -80°C until assayed.

### 2.4.3 Fresh homogenate Betong watercress juice preparation

Fresh Betong watercress was purchased from a local farm in Betong district, Yala province, Thailand. Then 150 g of Betong watercress was cleaned, chopped and added with 100 mL of deionized water. Mixture was blended for 1 min into a fine paste using a kitchen blender and filtered by filter cloth prior to store at -80°C (Jiao *et al.*, 1998). After filtration, the total volume of Betong watercress juice (210 mL) is collected. Therefore, concentration of fresh Betong watercress is 714.28 mg/mL.

### 2.4.4 Cooked homogenate Betong watercress juice preparation

The cooked homogenate Betong watercress preparation was described below. Fresh raw Betong watercress 150 g and deionized water 100 mL were placed in a microwave and cooked (Light-Up Dial®) for 15 min at full power (800 watts). Cooked Betong watercress was blended for 1 min into a fine paste using a kitchen blender and

filter by filter cloth prior to store at -80$^{o}$C (Jiao *et al.*, 1998). The total volume of cooked Betong watercress juice (190 mL) is collected. Therefore, concentration of cooked Betong watercress is 789.47 mg/mL.

### 2.4.5 Determination of glutathione *S*-transferases

**Principles**

Glutathione *S*-transferases catalyze the reaction of such compounds with the –SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble. The glutathione *S*-transferases activity was determined by a spectrophotometric method by the reaction between the standard substrate (1-chloro-2,4-dinitrobenzene, CDNB) and co-substrate (reduced glutathione, GSH) (Habig *et al.*, 1974).

The mechanism of glutathione to the CDNB, reduced glutathione is ionized to form proton ($H^+$) and thiolate anion $[GS]^-$. And then nucleophilic combined thiolate anion on C-1 of the aromatic nucleus; formation of mesenheimer complex and of 1-(*S*-glutathionyl)-2,4-dinitrobenzene (GS-DNB) followed at 340 nm (Figure 2.2) ( Van der Aar *et al.*, 1996; Habdous *et al.*, 2002).

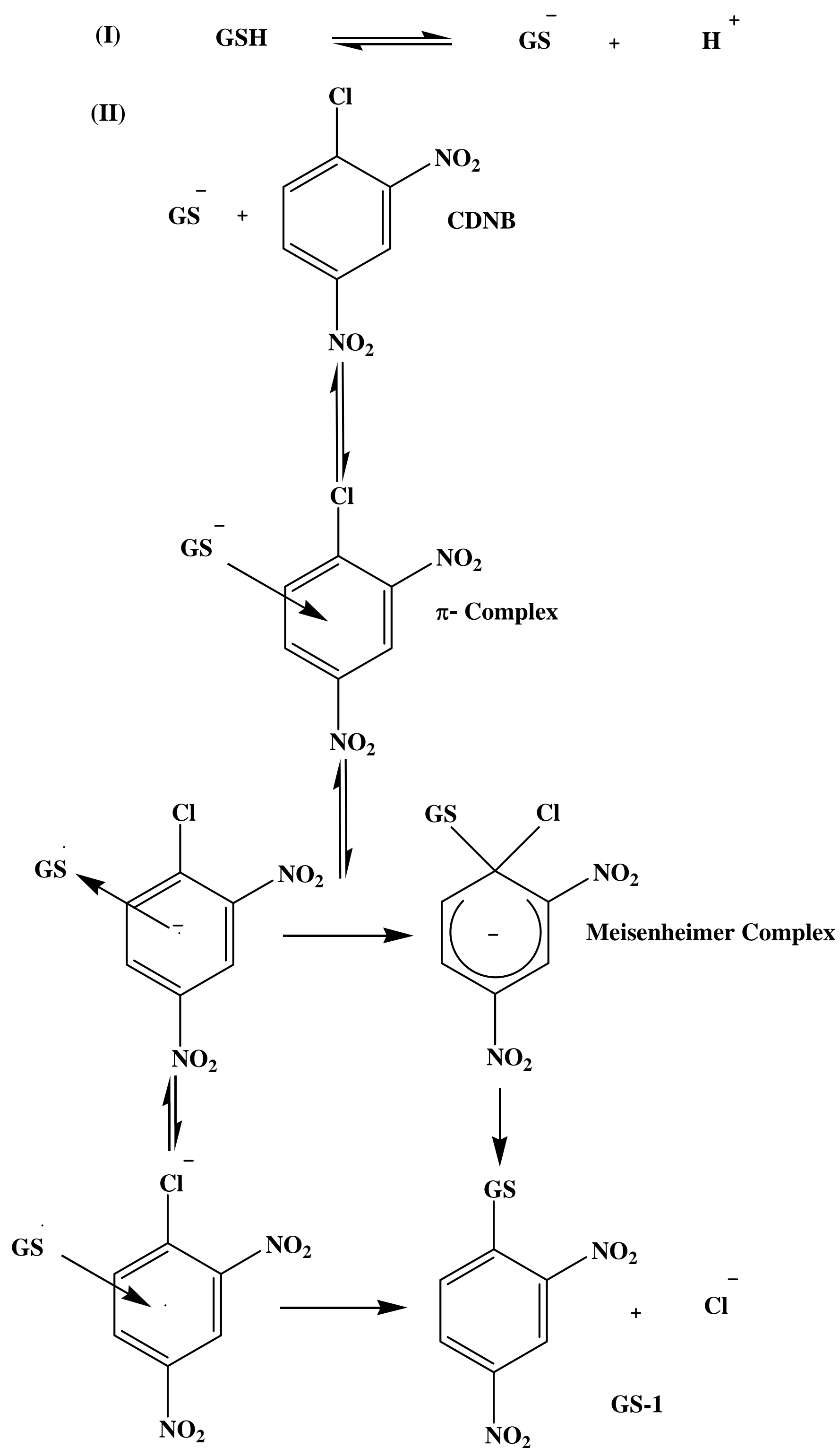



**Figure 2.2** The mechanism of glutathione to the CDNB (Van der Aar *et al.*, 1996)

**Procedure**

Glutathione *S*-transferases specific activity was measured according to the procedure of Habig *et al* (1974). This procedure is described as the following.

Enzymatic activity was monitored by measuring the conjugation of CDNB with GSH. One mL of enzyme assay was prepared as followed. First, the mixture of 100 μL of 1mM GSH and 100 μL of 1mM CDNB were prepared. Next, the mixture was added with 700 μL of 0.1 M potassium phosphate buffer at a pH 6.5. The reaction was initiated by the addition of sample. The increase in absorbance at 340 nm was determined using spectrophotometer. The formation of GS-DNB conjugation was recorded for 5 minutes, at 30-seconds interval.

The GST activity was calculated using the extinction coefficient of 9.6 $mM^{-1}$ $cm^{-1}$. One unit of glutathione *S*-transferases is represented by d μmol/mL/min at 25°C**.** Blank consisting were buffer, GSH, CDNB, without tissues homogenate in a total volume of 1 mL.

Glutathione *S*-transferase activity calculation = $$\frac{\Delta \text{absorbance } 340/\text{min} \times \text{Vt/Vs}}{0.0096\ \mu\text{M}} \times \text{d.f.}$$

Where Vt = total volume (mL)

Vs = sample volume (mL)

d.f = dilution factor

**2.4.6 Bradford protein assay**

**Principles**

The Bradford assay is a protein determination method that involves with binding of Coomassie Brillian Blue G-250 dye to proteins. After forming a complex, dry-protein binding, the complex which is a stable unprotonated form, was detected by using spectrophotometer at 595 nm. (Bradford, 1976).

**Procedure**

The determination of protein content in liver samples was carried out as previously described by Bradford (1976) with some modifications as the following.

A standard curve with bovine serum albumin (BSA) as the protein standard was made of the readings of six different dilutions of 1 mg/mL protein standard solution. Final concentrations were 1.25, 2, 3, 5, 7 and 10 μg/mL. Bradford solution (coomassie blue) was diluted with water (1:5). Then 800 μL of different concentration of BSA or samples (1:800) and 200 μL of bradford reagent was mixed to the test tube. Absorbance of standard and sample was measured with a spectrophotometer for 5 minute at 595 nm. Protein content was quantified from a standard curve plotted with different concentrations of BSA and values were presented as mg/mL.

**2.4.7 Determination of reduced glutathione**

The reduced glutathione was obtained from subtraction of total glutathione with oxidized glutathione, as described below.

**2.4.7.1 Assay of total glutathione**

**Principles**

The total glutathione (GSH and GSSG) was determinated by DTNB-GSSG reductase recycling assay (Griffith, 1980). The GSH is oxidized by DTNB (5,5'-dithio-bis-2-(nitro benzoic acid)) or Ellman's reagent to produced GSSG with 5-thio-2-nitro-benzoic acid ($TNB^-$), which is a yellow color. The GSSG is reduced to GSH by the action of the highly specific GSSG reductase (GR) and its cofactor NADPH and GSH reacts with DTNB again to give $TNB^-$. The reaction are as follows (Figure 2.3) (Araujo *et al.*, 2008)

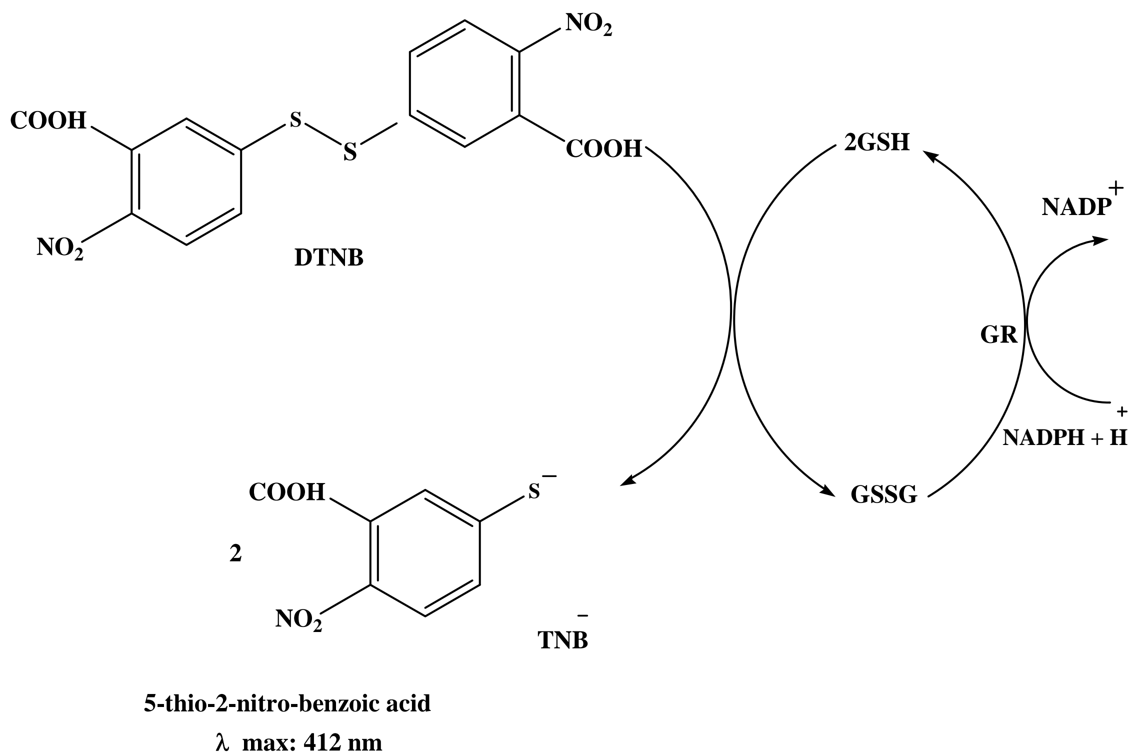



**Figure 2.3** The DTNB-GSSG reductase recycling system for total glutathione (Araujo *et al.*, 2008).(GR: Glutathione reductase)

**Procedure**

Total glutathione concentration was measured according to the procedure of Griffith (1980), as described below. Firstly, 700 μL of daily buffer (0.248 mg/mL NADPH in stock buffer; 143 mM sodium phosphate buffer plus 6.3 mM $Na_4EDTA$, pH 7.5) was mixed with 100 μL of 6 mM DTNB, and 190 μL of distilled water. Second, then incubated at 30$^{o}$C in water bath for 15 minutes. Finally, added 10 μL of sample or glutathione standard for standard curve and 266 U/mL glutathione reductase (GR) to initiate the reaction. The absorbance was measured every 30 seconds during 5 minutes at 412 nm with the spectrophotometer. The amount of glutathione in each sample was determined by comparison with standard curve, which is plotted against the rate of

change versus time ($\Delta A412\ min^{-1}$) with difference concentration of glutathione standard (1.56 – 50 μM). The results were expressed as μmole/g liver.

**2.4.7.2 Assay of oxidized glutathione**

**Principles**

The determination of oxidized glutathione (GSSG) in biological sample is often difficult because GSSG is normally present at very low levels as compared to GSH. The GSSG were determinated by DTNB-GSSG reductase recycling assay and using the 2-vinylpyridine (2VP) as thiol-blocking reagent were masked glutathione. The reaction is shown in figure 2.4.

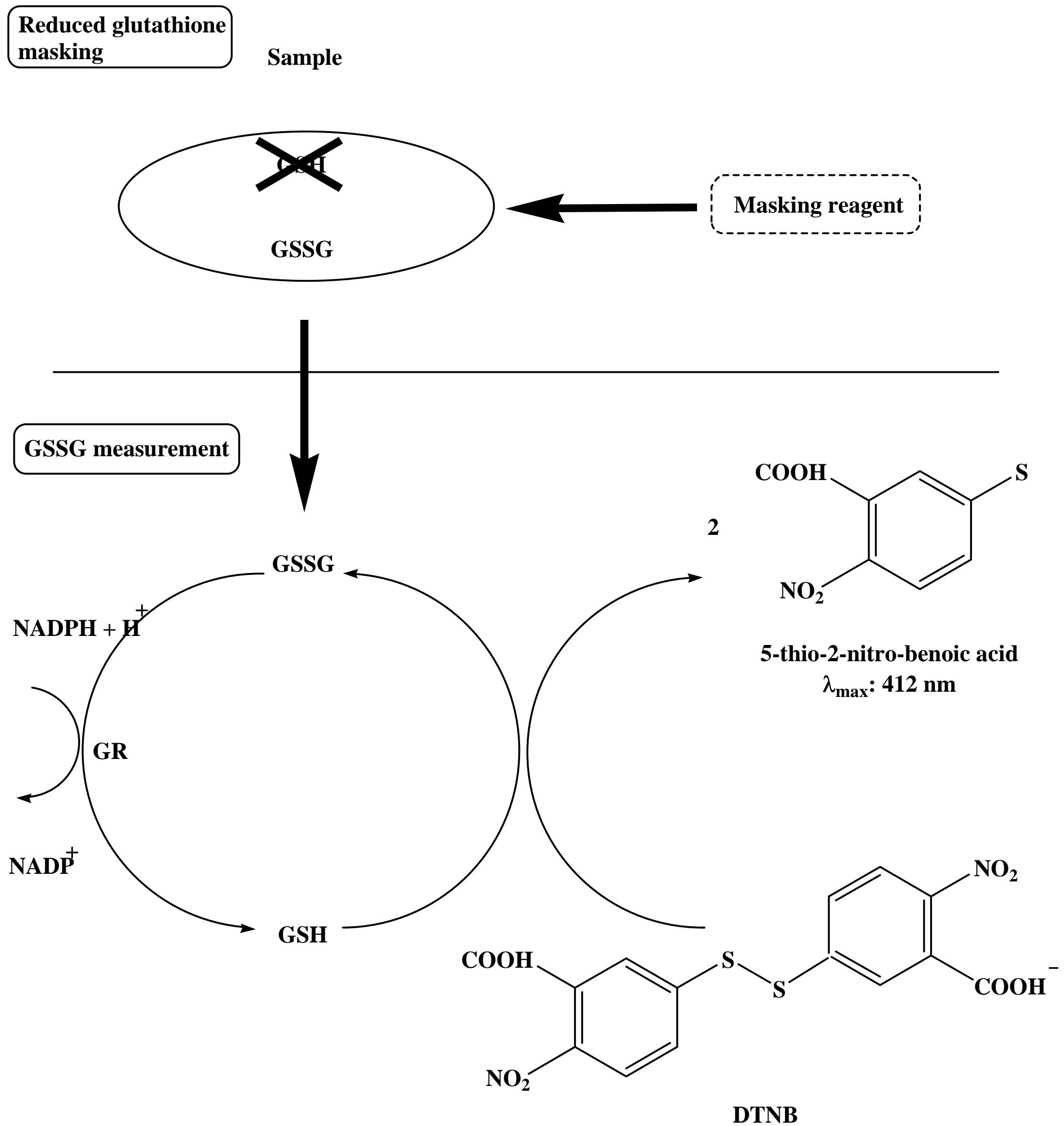



**Figure 2.4** The DTNB-GSSG reductase recycling system for oxidized glutathione (GSSG) (Dojindo, 2003). (GR: Glutathione reductase)

**Procedure**

The GSSG determination is based on an enzymatic recycling assay of DTNB-GSSG reductase (Griffith, 1980).

First of all, the derivatizations of liver sample as described below. The addition of 100 µL the 5% sulfosalicylic acid (SSA) supernatant solution was prepared in a 1.5 mL plastic tube. 2 µL of 2VP and 4 µL of triethanolamine (TEA) were added into the test tube. Then the solution is vigorously mixed. The final pH should be between 6 and 7 after incubated at room temperature for 60 minutes.

Finally, the mixture was added 700 µL of daily buffer, 100 µL of 6 mM DTNB and 190 µL of distilled water, and then with incubated at $30^{o}$C for 15 minutes. 10 µL derivatized sample and 266 U/mL glutathione reductase (GR) was added to initiate the assay. The increasing absorbance was monitored every 30 seconds for 5 minutes at 412 nm using spectrophotometer.

The rate of 5-thio-2-nitrobenzoic acid (TNB) formation, which is proportional to the content of GSSG in the sample was determined by comparison with standard curve by plotting between rate of change versus time ($\Delta$A412 $min^{-1}$) with difference concentration of oxidized glutathione standard. The results were expressed as µmole/g liver.

**2.5 Statistical Analysis**

All values are expressed as mean ± standard error (X±SEM). GST activity was statistically assessed by Kruskal-Wallis test followed by Mann-Whitney U test for differences between groups.

The glutathione levels were statistically assessed type of data distribution by One Sample Kolmogorov-Smirnov test. Data were analyzed using one-way analysis of variance (ANOVA) to test for the equality of group means and LSD for multiple comparisons. Differences at a *p*-value of less than 0.05 were considered as the level of significance in all statistical tests.

# CHAPTER 3

# Results

### 3.1 Effects of PEITC, fresh and cooked Betong watercress on GST activity in rats.

The effects of PEITC, fresh Betong watercress (FBWC) and cooked Betong watercress (CBWC) on GST activity in rat's liver were investigated. The activity of GST was determined after 3 day of treatment. A summary of this enzyme activity base on treatment was given in table 3.1.

The results showed that mean GST activity (µmol/mL/min) were 149.33 ± 8.74; in deionized water treated group, 158.02 ± 3.65; in corn oil treated group, 184.79 ± 3.90; in 25 mg/kgBW PEITC, 209.21 ± 1.61 ; in 50 mg/kgBW PEITC, 239.17 ± 8.67 ; in 100 mg/kgBW PEITC, 235.00 ± 36.38 ; in 357 mg/kgBW FBWC, 231.63 ± 8.17 ; in 714 mg/kgBW FBWC, 288.73 ± 35.40; in 1428 mg/kgBW FBWC, 254.95 ± 30.76; in 357 mg/kgBW CBWC, 313.91 ± 40.97; in 714 mg/kgBW CBWC and 295.56 ± 40; in 1428 mg/kgBW CBWC.

The activity of GST in 25, 50, and 100 mg/kg PEITC were significantly increased when compared with corn oil treated group as shown in Fig 3.1. In addition varying doses of PEITC were also increased GST activity. GST activity in 50 mg/kg PEITC was significantly increased when compared with 25 mg/kg PEITC. GST activity in 100 mg/kg PEITC was significantly increased when compared with 25 and 50 mg/kg PEITC. The activity of GST in 714 mg/kgBW FBWC was significantly increased when compared with deionized water treated group. GST activity in 1428 mg/kgBW FBWC was significantly increased when compared with deionized water treated group and 714 mg/kgBW FBWC. The activity in 357, 714, 1428 mg/kgBW CBWC were significantly increases when compared with deionized water treated group.

**Table 3.1** Effect of PEITC, fresh and cooked Betong watercress on glutathione *S*-transferase activity in rats.

| Group /dose | DI control | Corn oil | PEITC | | | FBWC | | | CBWC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 mL/kg | 10 mL/kg | 25 mg/kg | 50 mg/kg | 100 mg/kg | 357 mg/kg | 714 mg/kg | 1428 mg/kg | 357 mg/kg | 714 mg/kg | 1428 mg/kg |
| | **GST activity (µmol/mL/min)** | | | | | | | | | | |
| mean | 149.33 | 158.02 | 184.79[**] | 209.21[**,#] | 239.17[**,#,##] | 235.00 | 231.63[*] | 288.73[*,†] | 254.95[*] | 313.91[*] | 295.56[*] |
| % increases | | | 16.9 | 32.4 | 51.4 | 57.4 | 54.7 | 93.4 | 70.7 | 110.2[a,c] | 97.9[b] |
| min | 121.67 | 147.14 | 173.33 | 203.96 | 209.38 | 119.79 | 219.38 | 241.25 | 208.33 | 235.83 | 214.79 |
| max | 174.58 | 165.10 | 196.88 | 212.92 | 258.33 | 323.13 | 262.08 | 393.66 | 339.79 | 416.67 | 386.48 |
| SD | 19.54 | 8.15 | 8.72 | 3.59 | 19.38 | 81.35 | 18.27 | 70.81 | 61.51 | 81.94 | 81.09 |
| SEM | 8.74 | 3.65 | 3.90 | 1.61 | 8.67 | 36.38 | 8.17 | 35.40 | 30.76 | 40.97 | 40.55 |
| n | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 |

Value are presented as mean ± SEM

* $P<0.05$ compared with DI control group by Kruskal-Wallis test followed by Mann-Whitney U.

** $P<0.05$ compared with corn oil treated group by Kruskal-Wallis test followed by Mann-Whitney U.

\# $P<0.05$ compared with 25 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

\## $P<0.05$ compared with 50 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

† $P<0.05$ compared with 714 mg/kgBW FBWC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

a $P<0.05$ compared with control group using one sample t-test.

b $P<0.05$ compared with 357 mg/kgBW CBWC treated group using one sample t-test..

c $P<0.05$ compared with 1428 mg/kgBW CBWC treated group using one sample t-test..

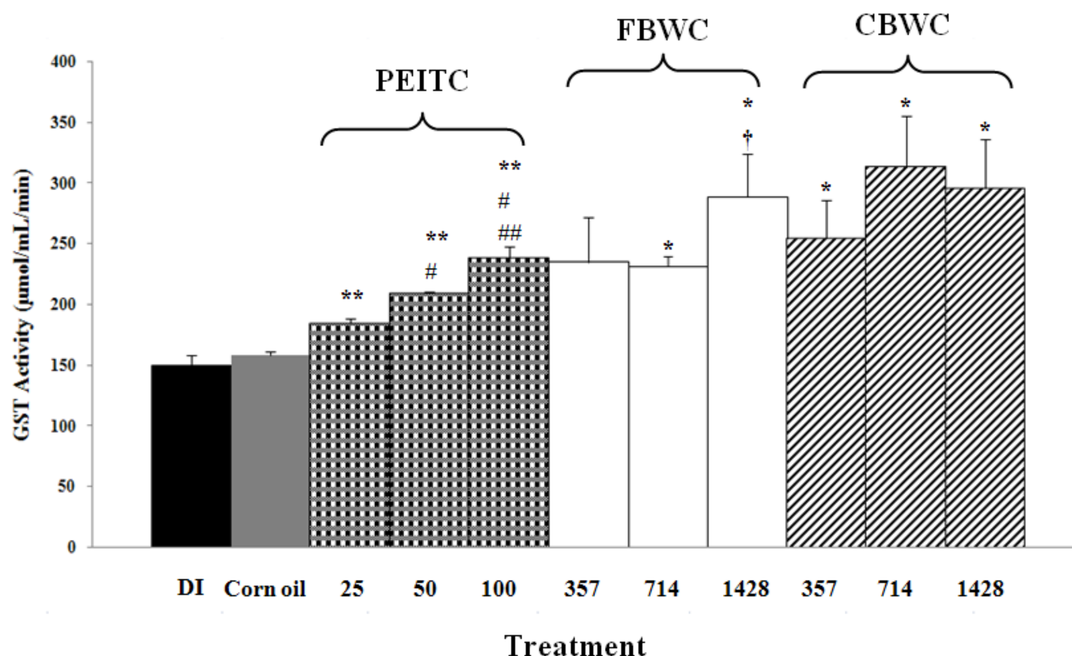



**Figure 3.1** GST activity after 3 days simultaneously oral administration of deionized water (DI 10 mL/kg), corn oil (10 mL/kg), phenethyl isothiocyanate (25, 50 and 100 mg/kgBW PEITC), fresh Betong watercress (357, 714 and 1428 mg/kgBW FBWC) and cooked Betong watercress (357, 714 and 1428 mg/kgBW CBWC). Significant differences were considered at $p<0.05$ (mean±SEM).

* $P<0.05$ compared with DI control group by Kruskal-Wallis test followed by Mann-Whitney U.

** $P<0.05$ compared with corn oil treated group by Kruskal-Wallis test followed by Mann-Whitney U.

# $P<0.05$ compared with 25 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

## $P<0.05$ compared with 50 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

† $P<0.05$ compared with 714 mg/kgBW FBWC by Kruskal-Wallis test followed by Mann-Whitney U.

**3.2 Effect of PEITC, fresh and cooked Betong watercress on protein content in rats.**

The effects of PEITC, fresh Betong watercress (FBWC) and cooked Betong watercress (CBWC) on protein content in rat's liver were investigated. The content of protein was determined after treated with various treatments for 3 days. A summary of this enzyme activity base on treatment was given in table 3.2.

The results showed that mean protein content (mg/mL) were 6.95 ± 0.16; in deionized water treated group, 4.95 ± 0.49; in corn oil treated group, 5.25 ± 0.34; in 25 mg/kgBW PEITC, 6.14 ± 0.45 ; in 50 mg/kgBW PEITC, 7.14 ± 0.52; in 100 mg/kgBW PEITC, 7.22 ± 0.77 ; in 357 mg/kgBW FBWC, 6.38 ± 0.26 ; in 714 mg/kgBW FBWC, 6.91 ± 0.39; in 1428 mg/kgBW FBWC, 6.12 ± 0.51 in 357 mg/kg BW CBWC, 6.28 ± 1.18; in 714 mg/kgBW CBWC and 7.00 ± 0.37; in 1428 mg/kgBW CBWC.

The protein content in corn oil was significantly increased when compared with deionized water treated group. Protein content in 100 mg/kgBW PEITC treated group was significantly increased when compared with corn oil treated group. The protein content in 100 mg/kgBW PEITC was significantly increased when compared with 25 mg/kgBW PEITC.

**Table 3.2** Effect of PEITC, fresh and cooked Betong watercress on protein content in rats.

| Group | Treatment | | Protein content (mg/mL) |
|---|---|---|---|
| I | DI control (mL/kgBW) | 10 | 6.95 ± 0.16 |
| II | Corn oil (mL/kgBW) | 10 | 4.94 ± 0.49* |
| III | | 25 | 5.25 ± 0.34 |
| IV | PEITC (mg/kgBW) | 50 | 6.14 ± 0.45 |
| V | | 100 | 7.14 ± 0.52**, # |
| VI | | 357 | 7.22 ± 0.77 |
| VII | FBWC (mg/kgBW) | 714 | 6.38 ± 0.26 |
| VIII | | 1428 | 6.91 ± 0.39 |
| IX | | 357 | 6.12 ± 0.51 |
| X | CBWC (mg/kgBW) | 714 | 6.28 ± 1.18 |
| XI | | 1428 | 7.00 ± 0.37 |

Value are presented as mean ± SEM

* $P<0.05$ compared with control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

# $P<0.05$ compared with 25 mg/kg BW PEITC using ANOVA followed by post hoc LSD.

**3.3 Effect of PEITC, fresh and cooked Betong watercress on total glutathione levels in rats.**

The effects of PEITC, fresh Betong watercress (FBWC) and cooked Betong watercress (CBWC) on total glutathione levels in rat's liver were investigated. The levels of total glutathione were determined after treated with various treatments for 3 days. A summary of this enzyme activity base on treatment was given in table 3.3.

The results showed that mean total glutathione levels (mM) were 1.20 ± 0.17; in deionized water treated group, 1.22 ± 0.08; in corn oil treated group, 0.78 ± 0.15; in 25 mg/kg BWPEITC, 1.22 ± 0.33 ; in 50 mg/kgBW PEITC, 0.80 ± 0.30 ; in 100 mg/kgBW PEITC, 1.39 ± 0.28 ; in 357 mg/kgBW FBWC, 1.06 ± 0.15 ; in 714 mg/kg BWFBWC, 1.71 ± 0.36; in 1428 mg/kgBW FBWC, 1.89 ± 0.46; in 357 mg/kgBW CBWC, 1.36 ± 0.16; in 714 mg/kgBW CBWC and 1.50 ± 0.13; in 1428 mg/kgBW CBWC.

The total glutathione levels in 25, 50, and 100 mg/kgBW PEITC were not significantly when compared with corn oil treated group as shown in Fig 3.2. In addition, the total glutathione levels in 357 mg/kgBW CBWC treated group was significantly increased when compared with714 mg/kgBW FBWC treated group.

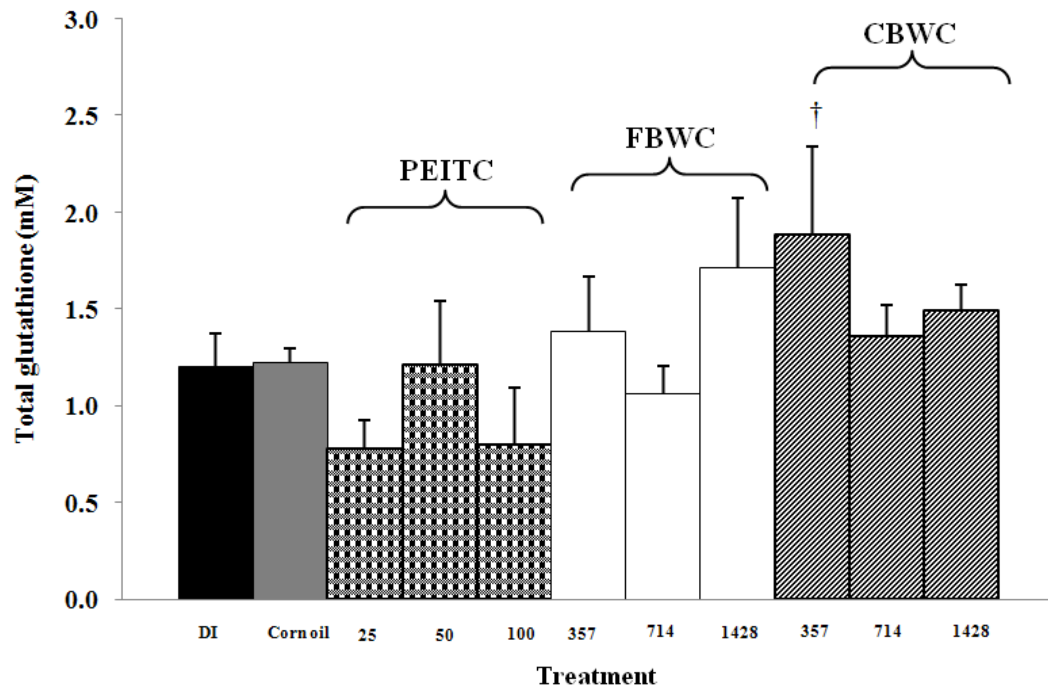



**Figure 3.2** The total glutathione levels after 3 days simultaneously oral administration of deionized water (DI 10 mL/kg), corn oil (10 mL/kg), phenethyl isothiocyanate (25, 50 and 100 mg/kgBW PEITC), fresh Betong watercress (357, 714 and 1428 mg/kg BW FBWC) and cooked Betong watercress (357, 714 and 1428 mg/kgBW CBWC). Significant differences were considered at $p<0.05$ (mean±SEM).

† $p<0.05$ compared with FBWC 714 mg/kg using ANOVA followed by post hoc LSD.

**3.4 Effect of PEITC, fresh and cooked Betong watercress on oxidized glutathione levels in rats.**

The effects of PEITC, fresh Betong watercress (FBWC) and cooked Betong watercress (CBWC) on oxidized glutathione levels in rat's liver were investigated. The levels of oxidized glutathione were determined after treated with various treatments for 3 days. A summary of oxidized glutathione was base on treatment given in table 3.3.

The results showed that mean oxidized glutathione levels (mM) were 0.49 ± 0.05; in deionized water treated group, 0.49 ± 0.04; in corn oil treated group, 0.2 ± 0.04; in 25 mg/kgBW PEITC, 0.33 ± 0.07 ; in 50 mg/kgBW PEITC, 0.15 ± 0.02 ; in 100 mg/kgBW PEITC, 0.28 ± 0.02 ; in 357 mg/kgBW FBWC, 0.36 ± 0.07 ; in 714 mg/kgBW FBWC, 0.24 ± 0.03; in 1428 mg/kgBW FBWC, 0.29 ± 0.04; in 357 mg/kgBW CBWC, 0.37 ± 0.04; in 714 mg/kgBW CBWC and 0.34 ± 0.05; in 1428 mg/kgBW CBWC.

The oxidized glutathione levels in 25, 50, and 100 mg/kgBW PEITC were significantly decreased when compared with corn oil treated group as shown in Fig 3.3. The oxidized glutathione in 357 and 1428 mg/kgBW FBWC were significantly decreased when compared with deionized water treated group. In addition, the oxidized glutathione levels in 357 mg/kgBW CBWC treated group was significantly decreased when compared with deionized water treated group.

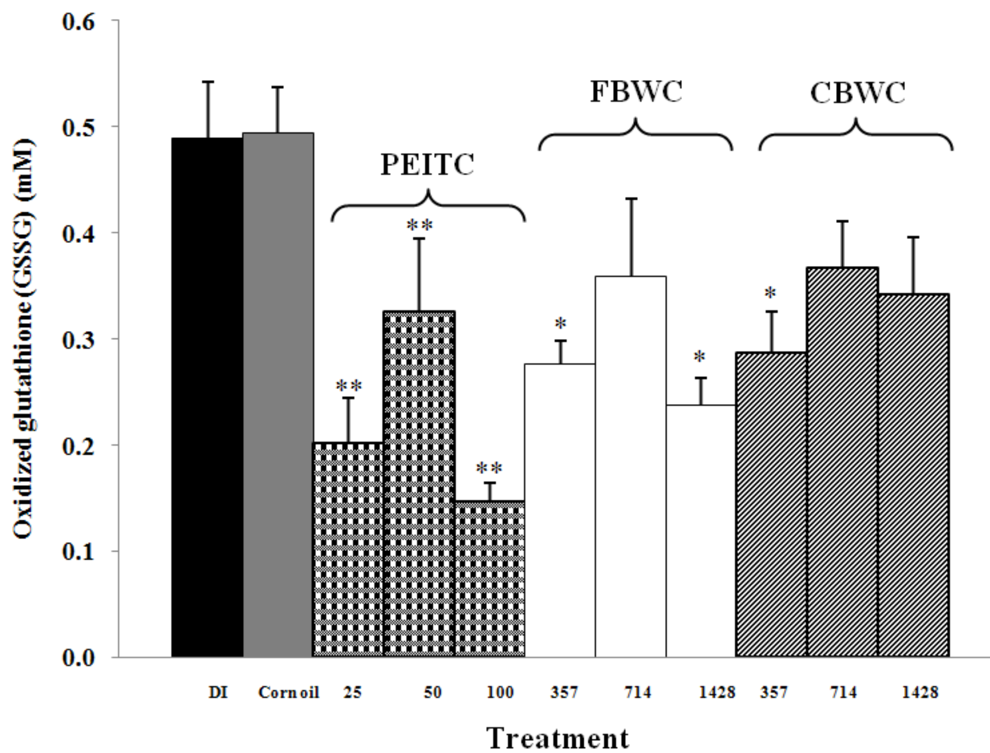



**Figure 3.3** The oxidized glutathione levels after 3 days simultaneously oral administration of deionized water (DI), corn oil, phenethyl isothiocyanate (25, 50 and 100 mg/kgBW PEITC), fresh Betong watercress (357, 714 and 1428 mg/kgBW FBWC) and cooked Betong watercress (357, 714 and 1428 mg/kgBW CBWC). Significant differences were considered at $p<0.05$ (mean±SEM).

* $p<0.05$ compared with DI control group using ANOVA followed by post hoc LSD.

** $p<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

**3.5 Effect of PEITC, fresh and cooked Betong watercress on reduced glutathione levels in rats.**

The effects of PEITC, fresh Betong watercress (FBWC) and cooked Betong watercress (CBWC) on reduced glutathione levels in rat's liver were investigated. The levels of reduced glutathione were determined after treated with various treatments for 3 days. A summary of this enzyme activity base on treatment was given in table 3.3.

The results showed that mean reduced glutathione levels (mM) were 0.71 ± 0.21; in deionized water treated group, 0.73 ± 0.09; in corn oil treated group, 0.58 ± 0.11; in 25 mg/kgBW PEITC, 0.89 ± 0.28 ; in 50 mg/kgBW PEITC, 0.65 ± 0.31 ; in 100 mg/kgBW PEITC, 1.11 ± 0.26 ; in 357 mg/kgBW FBWC, 0.70 ± 0.19 ; in 714 mg/kgBW FBWC, 1.48 ± 0.37; in 1428 mg/kgBW FBWC, 1.6 ± 0.45; in 357 mg/kgBW CBWC, 0.99 ± 0.19; in 714 mg/kgBW CBWC and 1.15 ± 0.16; in 1428 mg/kgBW CBWC.

The reduced glutathione levels in the PEITC treated group were not significantly different when compared with corn oil treated group as shown in Fig 3.4. The reduced glutathione levels in 1428 mg/kgBW FBWC treated group was significantly increased when compared with deionized water, 714 mg/kgBW FBWC treated group. In contrast, other treatments of FBWC treated group were not significantly different when compared with deionized water, 714 mg/kgBW FBWC treated group. The reduced glutathione levels in 357 mg/kgBW CBWC treated group was significantly increased when compared with deionized water and 714 mg/kgBW FBWC treated group. However, other treatments of CBWC treated group were not significantly different when compared with deionized water and 714 mg/kgBW FBWC treated group.

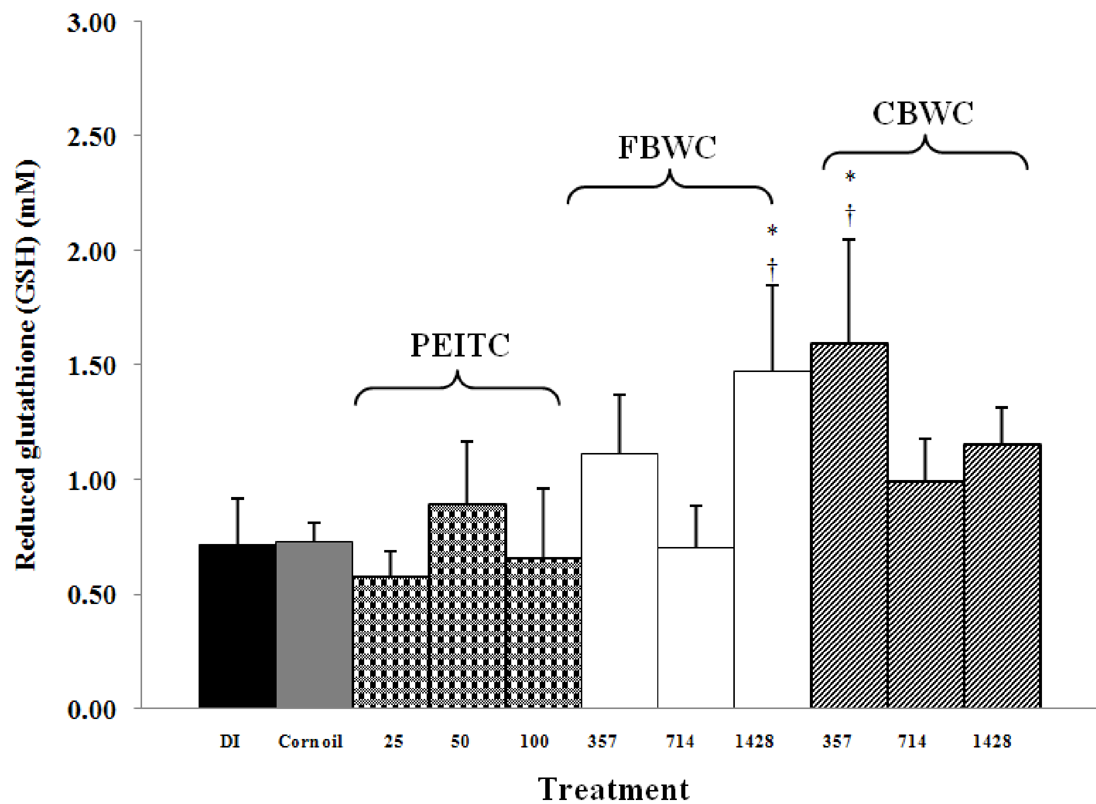



**Figure 3.4** The reduced glutathione levels after 3 days simultaneously oral administration of deionized water (DI), corn oil, phenethyl isothiocyanate (25, 50 and 100 mg/kgBW PEITC), fresh Betong watercress (357, 714 and 1428 mg/kgBW FBWC) and cooked Betong watercress (357, 714 and 1428 mg/kgBW CBWC). Significant differences were considered at $p<0.05$ (mean±SEM).

* $p<0.05$ compared with control group using ANOVA followed by post hoc LSD.

† $p<0.05$ compared with 714 mg/kgBW FBWC treated group using ANOVA followed by post hoc LSD.

**3.6 Effect of PEITC, fresh and cooked Betong watercress on oxidative status in rat's liver.**

The effects of PEITC, fresh Betong watercress (FBWC) and cooked Betong watercress (CBWC) on oxidative status in rat's liver were investigated. The oxidative status was determined from the GSH/GSSG ratio. The GSH/GSSG ratio was calculated by dividing the difference between the reduced glutathione and oxidized glutathione after treated with various treatments for 3 days. A summary of this enzyme activity base on treatment was given in table 3.3.

The results showed that mean GSH/GSSG were 1.63 ± 0.63; in deionized water treated group, 1.56 ± 0.35; in corn oil treated group, 3.03 ± 0.55; in mg/kgBW PEITC 25, 3.10 ± 0.70; in 50 mg/kgBW PEITC, 5.32 ± 2.60; in 100 mg/kgBW PEITC, 3.92 ± 0.69; in 357 mg/kgBW FBWC, 2.47 ± 0.71; in 714 mg/kgBW FBWC, 6.62 ± 1.97; in 1428 mg/kgBW FBWC, 5.72 ± 1.38; in 357 mg/kgBW CBWC, 2.92 ± 0.76; in 714 mg/kgBW CBWC and 3.80 ± 1.05; in 1428 mg/kgBW CBWC.

The GSH/GSSG ratio of 100 mg/kgBW PEITC treated group was significantly different when compared with corn oil treated group as shown in Fig 3.5. The GSH/GSSG ratio of 1428 mg/kgBW FBWC treated group was significantly different when compared with deionized water and 714 mg/kgBW FBWC treated group. In addition, the GSH/GSSG ratio of 357 mg/kgBW CBWC treated group was significantly different when compared with deionized water.

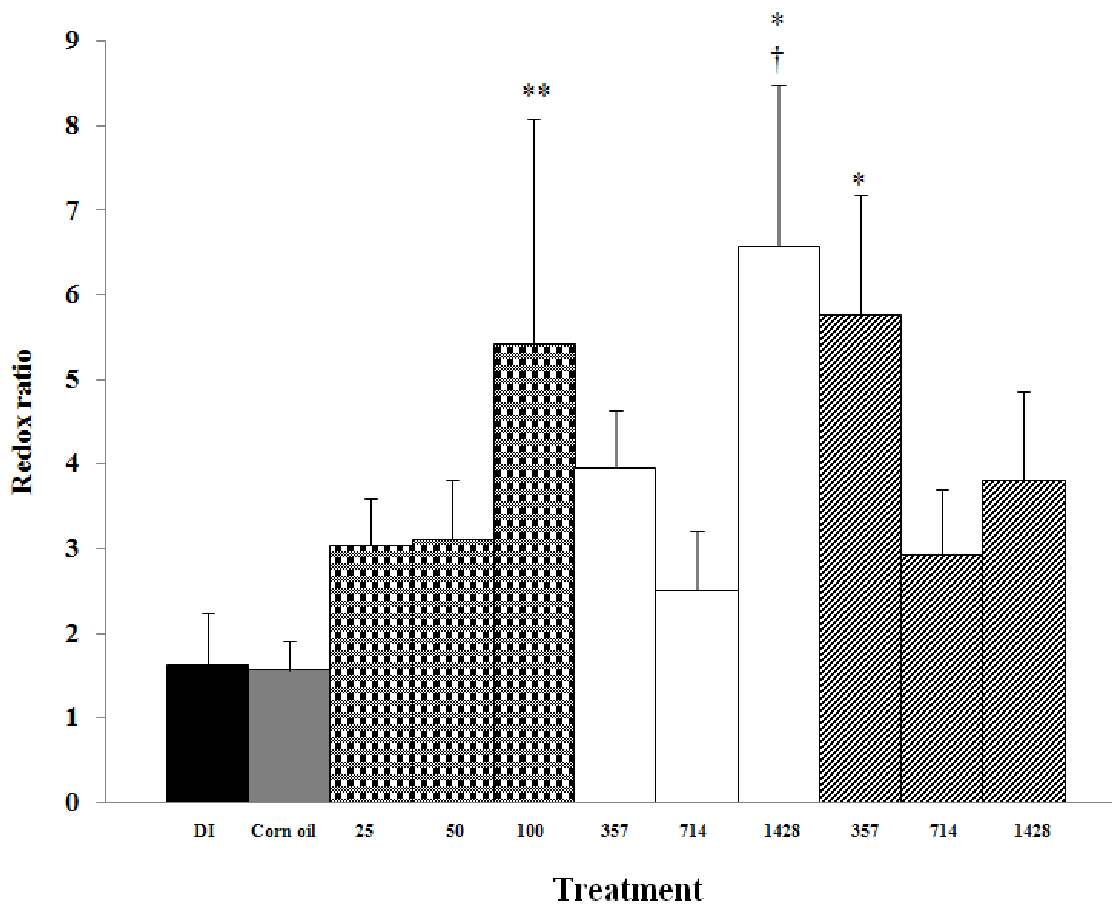



**Figure 3.5** The redox ratio after 3 days simultaneously oral administration of deionized water (DI), corn oil, phenethyl isothiocyanate (25, 50 and 100 mg/kgBW PEITC), fresh Betong watercress (357, 714 and 1428 mg/kgBW FBWC) and cooked Betong watercress (357, 714 and 1428 mg/kgBW CBWC). Significant differences were considered at $p<0.05$ (mean±SEM).

* $p<0.05$ compared with control group using ANOVA followed by post hoc LSD.

** $p<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

† $p<0.05$ compared with 714 mg/kgBW FBWC treated group using ANOVA followed by post hoc LSD.

**Table 3.3** Effect of PEITC, fresh and cooked Betong watercress on glutathione levels at 3 days in rats.

| Group | Treatment | Total glutathione (mM) | Reduced glutathione (GSH) (mM) | Oxidized glutathione (GSSG) (mM) | GSH/GSSG |
|---|---|---|---|---|---|
| I | Deionized water (n=4) | 1.2 ± 0.17 | 0.71 ± 0.21 | 0.49 ± 0.05 | 1.63 ± 0.63 |
| II | Corn oil (n=5) | 1.22 ± 0.08 | 0.73 ± 0.09 | 0.49 ± 0.04 | 1.56 ± 0.95 |
| III | 25 mg/kgBW PEITC (n=5) | 0.78 ± 0.15 | 0.58 ± 0.11 | 0.2 ± 0.04** | 3.03 ± 0.55 |
| IV | 50 mg/kgBW PEITC (n=5) | 1.22 ± 0.33 | 0.89 ± 0.28 | 0.33 ± 0.07**, ### | 3.1 ± 0.70 |
| V | 100 mg/kgBW PEITC (n=5) | 0.8 ± 0.30 | 0.65 ± 0.31 | 0.15 ± 0.02** | 5.32 ± 2.60** |
| VI | 357 mg/kgBW FBWC (n=4) | 1.39 ± 0.28 | 1.11 ± 0.26 | 0.28 ± 0.02* | 3.92 ± 0.69 |
| VII | 714 mg/kgBW FBWC (n=5) | 1.06 ± 0.15 | 0.7 ± 0.19 | 0.36 ± 0.07 | 2.47 ± 0.71 |
| VIII | 1428 mg/kgBW FBWC (n=4) | 1.71 ± 0.36 | 1.48 ± 0.37*, † | 0.24 ± 0.03* | 6.62 ± 1.97*,† |
| IX | 357 mg/kgBW CBWC (n=4) | 1.89 ± 0.46† | 1.6 ± 0.45*, † | 0.29 ± 0.04* | 5.72 ± 1.38* |
| X | 714 mg/kgBW CBWC (n=4) | 1.36 ± 0.16 | 0.99 ± 0.19 | 0.37 ± 0.04 | 2.92 ± 0.76 |
| XI | 1428 mg/kgBW CBWC (n=4) | 1.5 ± 0.13 | 1.15 ± 0.16 | 0.34 ± 0.05 | 3.8 ± 1.05 |

Value are presented as mean±SEM

* $P<0.05$ compared with DI control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

### $P<0.05$ compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† $P<0.05$ compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.

# CHAPTER 4

## Discussion

Many studies on the effects of phytochemicals including PEITC in watercress on enzyme in phase II of drug metabolism were suggested that PEITC should induce GST.

The results indicated that PEITC is capable of inducing the activities of GST. The increased activity of GST in rat liver was seen after oral administration of PEITC for 3 days. The induction of GST activity by PEITC was demonstrated in accordance with the results of Guo and colleagues (1992) which reported that 1 mmol/kg PEITC (163 mg/kg BW) elevated GST activity in rat.

In addition, this study is consistence with the previous studied by Seo and colleagues (2000) who reported that treatment with 3.1 and 31.6 mg/kg BW PEITC in rat tended to increase GST activity. Moreover, 100 and 200 mg/kg BW PEITC in rat for 3 days showed 1.6 and 1.8 fold increases in liver GST.

In this study, the percentage of increase in GST activity in rats after receiving of a multiple dose of 25, 50 and 100 mg/kgBW PEITC for 3 days were not significantly by approximately 16.9 % when compared with corn oil treated group. The highest percentage of increase was 51.4 % after exposure to 100 mg/kg BW PEITC. An increase in enzyme activity by PEITC was dose - dependent. The previous studies found that single dose of oltipraz (4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione), an antischistosomal drug, and 1, 7-phenanthroline showed a strong induction of alpha GST mRNA in rat liver between 12 and 18 h after administration (Lamp and Franklin, 2000). The early study of GST stimulation by organic compounds in liver has been described at 18-24 h after administration (Lampe and Peterson, 2002; Cho and Kim, 2000; Lamb and Franklin, 2000). In addition, the previous studied showed that 4-methylthiazole elevated alpha class of GST i.e. rGSTA2, A3, A5 by increase mRNAs in the liver at 24 h after treatment, while level of alpha class rGSTA was increased after 3 days of treatment.

However, other types of thiazole were minimally activate GST such as 5-methyl-, dimethyl- and trimethyl-substituted thiazoles.

In the present study, the effect of FBWC on GST activity was compared with deionized water treated group. The percentage of GST activity increasing in rats after received multiple doses of 357, 714, 1428 mg/kg BW FBWC was approximately 54.7 – 93.4 % when compared with deionized water treated group. The activity of GST in FBWC treated groups were slightly increased depend on dose but these levels were not significantly different. This study demonstrated that FBWC juice contain amount of PEITC which is released by myrosinase-mediated hydrolysis when raw vegetables are chopped or chewed. In addition, bacteria in large intestine are also to converse glucosinolates to PEITC.

The present study, the effect of CBWC on GST activity was also study. The results shown that the percentages of increasing of GST activity for 357mg/kgBW CBWC for 3 days was not significantly increased approximately 70.7 % when compared with deionized water treated group. However, the percentages of increasing of GST activity in 714 mg/kgBW CBWC was significantly increased approximately 110.2% when compared with deionized water treated group and 1428 mg/kgBW FBWC treated group. In addition, the percentages of increasing of GST activity in 1428 mg/kgBW CBWC were significantly increased approximately 97.9 % when compared with 714 mg/kgBW CBWC treated group. The GST activity in FBWC treated rat and CBWC treated rat was not difference. This result suggested that rat also convert glucosinolate into PEITC after ingestion of cooked Batong watercress as the CBWC used in this study was completely devoid of myrosinase activity by heating with microwave. Therefore, these results indicated that the extent of conversion was similar after ingesting uncooked. However, in the previous studied by Getahun and Chung (1999) they found that the extent of conversion glucosinolate after ingestion fresh watercress was more than that after ingestion uncooked.

However, there was no clear correlation of the increases in GST activity between FBWC and CBWC treated group. The results showed that the percentage of increase of GST activity for all CBWC treated group were much higher than that of FBWC treated group. In fact, the GST activity of FBWC was higher than

that of CBWC. This could be due to the amount of PEITC compound in FBWC in higher than in CBWC.

In the study shown that the increase in GST activity was paralleled with the rising in liver protein content in PEITC and Betong watercress treated groups. But this study did not measure the targeted protein. Therefore, the type of protein which was increased did not state here. However, Seo and colleague (2000) have been reported that PEITC-treated rat at 31.6, 100, 200 mg/kg increased hepatic GST protein levels and correlate with the increases of GST mRNA levels.

The mechanism of GST induction after PEITC treatment is supposed to induce drug metabolizing enzymes through mitogen-activated protein kinase (MAPK) pathway and nuclear factor erythroid 2-related factor 2 (Nrf2) (Hayes *et al.*, 2005). Nguyen and colleagues was demonstrated the mechanism of GST induction via increased nuclear translocation of Nrf2 (Nguyen *et al.*, 2009).

The effects of PEITC, FBWC, CBWC on glutathione levels were also studied. Total glutathione is a summation of GSH and GSSG. Therefore the levels of GSH and GSSG were considered. In the present study, reduced glutathione levels for all of PEITC treated group were slightly decreased, when compared with all of corn oil treated group. Reduced glutathione concentration was not significantly different from corn oil treated group. But the redox ratio (GSH/GSSG) was increased due to the depletion of oxidized GSH in liver. This evidence is resulting in decrease oxidative stress to cell. The results was not shown before in the previous studied. Depletion of reduced glutathione may be due to the level of glutathione did not recover after exposure to PEITC for 3 days. Previously, Ye and Zhang (2001) examined the effect of the duration of exposure to dietary isothiocyanate (ITC) ; allyl-ITC, benzyl-ITC, phenethyl-ITC and sulforaphane [1-isothiocyanato-(4R,S)-(methylsulfinyl)butane], on the elevation of glutathione levels in mouse skin papilloma cells by incubating cells with 5 µM of ITC for 12, 24, 48 or 72 h. They found that cellular glutathione level was highest after incubation with each type of ITC for 24 h which correlated with the highest of the area under time-concentration curve (AUC) of all-ITC in PE cell. However, as studied in human myeloblastic leukaemia ML-1 cells and human leukaemia cells HL60 cellular GSH concentration were initial decreased and recover after 12 h and declined again after 24 h (Xu and

Thornalley, 2001) In addition, PEITC was also to lower mitochondrial glutathione also (Chen *et al.*, 2011) Redox status in FBWC treated group was similar to PEITC treated group. But redox state was not correlated well in CBWC treated group as the results shown that redox status in CBWC treated group was larger in low dose whereas smaller in high dose.

Glutathione, the most abundant low-molecular-weight thiol in mammalian cells, is display in reduced and oxidized forms. The reduced form of glutathione is 10 to 100 fold higher than the oxidized forms. An increase in intracellular GSSG can occur from the breakdown of $H_2O_2$ by glutathione peroxidase. Because of the relatively low concentration of oxidized form in the cell compared with reduced form, a minor elevation in the oxidation of reduced glutathione to oxidized glutathione can result in a significant elevation in intercellular GSSG levels (Klatt and Lamas, 2000; Finkel, 2000). Glutathione is an important metabolite in living organism that role not only in detoxifying other xenobiotics, but in many other processing such as redox control, protection against oxidative stress, protein synthesis, DNA synthesis and repair, amino acid transport and functioning of the immune system (Dickinson and Forman, 2002).

# CHAPTER 5

# Conclusion

Cruciferous vegetable including watercress has been used for health promotion and reduce cancerous disease risk. Many types of ITCs in this vegetable have been shown the chemopreventive effect in animal studies by inhibited metabolizing enzyme in phase I and induce enzyme in phase II. As Betong water cress is a local vegetable in southern part of Thailand and widely consumed among the resident in the vicinity. Since there is very little information related to effects of Betong water cress on GST activity and GSH level in animal model. Therefore, the study was planned to detect the alteration of GST and GSH after orally administration of fresh and cooked Betong watercress. The results of this study were concluded as follows.

The multiple oral doses of PEITC, fresh and cooked Betong watercress increase glutathione *S*- transferase activities and elevate the rat liver protein content. Additionally, PEITC, FBWC and CBWC were increase the GSH/GSSG ratio, an important index of oxidative stress in cells. The elevation of redox ratio in liver by Betong watercress was greatly interesting. All of these results suggested that PEITC, FBWC and CBWC would enhance the detoxification process in phase II.

Furthermore, the consumption of cooked Betong watercress also produced the effect on GST activity, GSH content similar to the uncooked vegetable.

Therefore, Betong watercress have might important pharmacological substances which can be used for next studies.

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# APPENDIX

## APPENDIX A

Effect of PEITC and Betong watercress on GST activity (µmol/mL/min) in the liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight: Body weight ratio (%) | GST activity (µmol/mL/min) |
|---|---|---|---|---|---|---|
| I | Control (distilled water) | 1 | 235 | 8.3 | 3.53 | 121.67 |
| | | 2 | 214 | 9.6 | 4.49 | 157.08 |
| | | 3 | 235 | 9.8 | 4.17 | 174.58 |
| | | 4 | 237 | 8.9 | 3.76 | 151.67 |
| | | 5 | 243 | 10.2 | 4.20 | 141.67 |
| | Mean ± SE | | 2.33 ± 4.92 | 9.4 ± 0.34 | 4.03 ± 0.17 | 149.33 ± 8.74 |
| II | Corn oil | 1 | 219 | 10 | 4.57 | 147.14 |
| | | 2 | 217 | 8.7 | 4.01 | 164.32 |
| | | 3 | 221 | 9 | 4.07 | 165.10 |
| | | 4 | 208 | 8.6 | 4.13 | 151.56 |
| | | 5 | 231 | 9 | 3.90 | 161.98 |
| | Mean ± SE | | 219 ± 3.69 | 9.1 ± 0.25 | 4.14 ± 0.11 | 158.02 ± 3.65 |
| III | 25 mg/kgBW PEITC | 1 | 235 | 9.4 | 4.00 | 173.33 |
| | | 2 | 223 | 9.3 | 4.17 | 196.88 |
| | | 3 | 194 | 8.1 | 4.18 | 182.92 |
| | | 4 | 203 | 7.9 | 3.89 | 182.08 |
| | | 5 | 224 | 8.4 | 3.75 | 188.75 |
| | Mean ± SE | | 216 ± 7.51 | 8.6 ± 0.31 | 4.00 ± 0.08 | 184.79 ± 3.90** |

* *P*<0.05 compared with DI control group by Kruskal-Wallis test followed by Mann-Whitney U.

** *P*<0.05 compared with corn oil treated group by Kruskal-Wallis test followed by Mann-Whitney U.

# *P*<0.05 compared with 25 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

## *P*<0.05 compared with 50 mg/kgBW PEITC treated group using by Kruskal-Wallis test followed by Mann-Whitney U.

† *P*<0.05 compared with 714 mg/kgBW FBWC by Kruskal-Wallis test followed by Mann-Whitney U.

## APPENDIX A

Effect of PEITC and Betong watercress on GST activity (µmol/mL/min) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight: Body weight ratio (%) | GST activity (µmol/mL/min) |
|---|---|---|---|---|---|---|
| IV | 50 mg/kgBW PEITC | 1 | 239 | 11.5 | 4.81 | 211.88 |
| | | 2 | 230 | 8.7 | 3.78 | 203.96 |
| | | 3 | 217 | 8.1 | 3.73 | 209.79 |
| | | 4 | 222 | 9.7 | 4.37 | 212.92 |
| | | 5 | 206 | 7.8 | 3.79 | 207.50 |
| | Mean ± SE | | 223 ± 5.62 | 9.2 ± 0.67 | 4.10 ± 0.21 | 209.21 ± 1.61 **,# |
| V | 100 mg/kgBW PEITC | 1 | 218 | 9.1 | 4.17 | 209.38 |
| | | 2 | 211 | 8.2 | 3.89 | 232.08 |
| | | 3 | 220 | 9.4 | 4.27 | 252.50 |
| | | 4 | 215 | 9.9 | 4.60 | 258.33 |
| | | 5 | 215 | 8.9 | 4.14 | 243.54 |
| | Mean ± SE | | 216 ± 1.53 | 9.1 ± 0.28 | 4.22 ± 0.12 | 239.17 ± 8.67 **,#,## |
| VI | 357 mg/kgBW FBWC | 1 | 243 | 10.3 | 4.24 | 323.13 |
| | | 2 | 235 | 12.7 | 5.40 | 302.08 |
| | | 3 | 267 | 11.2 | 4.19 | 223.13 |
| | | 4 | 263 | 11.6 | 4.41 | 206.88 |
| | | 5 | 270 | 11.6 | 4.30 | 119.79 |
| | Mean ± SE | | 256 ± 7.81 | 11.5 ± 0.43 | 4.51 ± 0.25 | 235.00 ± 36.38 |

* *P*<0.05 compared with DI control group by Kruskal-Wallis test followed by Mann-Whitney U.

** *P*<0.05 compared with corn oil treated group by Kruskal-Wallis test followed by Mann-Whitney U.

# *P*<0.05 compared with 25 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

## *P*<0.05 compared with 50 mg/kgBW PEITC treated group using by Kruskal-Wallis test followed by Mann-Whitney U.

† *P*<0.05 compared with 714 mg/kgBW FBWC by Kruskal-Wallis test followed by Mann-Whitney U.

## APPENDIX A

Effect of PEITC and Betong watercress on GST activity (µmol/mL/min) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight: Body weight ratio (%) | GST activity (µmol/mL/min) |
|---|---|---|---|---|---|---|
| VII | 714 mg/kgBW FBWC | 1 | 218 | 9.1 | 4.17 | 235.42 |
| | | 2 | 211 | 8.2 | 3.89 | 262.08 |
| | | 3 | 220 | 9.4 | 4.27 | 221.04 |
| | | 4 | 215 | 9.9 | 4.60 | 219.38 |
| | | 5 | 215 | 8.9 | 4.14 | 220.21 |
| | Mean ± SE | | 216 ± 1.53 | 9.1 ± 0.28 | 4.22 ± 0.12 | 231.63 ± 8.17* |
| VIII | 1428 mg/kgBW FBWC | 1 | 247 | 11.9 | 4.82 | 393.66 |
| | | 2 | 244 | 10.8 | 4.43 | 267.92 |
| | | 3 | 274 | 12.8 | 4.67 | 252.08 |
| | | 4 | 258 | 11.5 | 4.46 | 241.25 |
| | Mean ± SE | | 256 ± 6.79 | 11.8 ± 0.42 | 4.59 ± 0.09 | 288.73 ± 35.40*,† |
| IX | 357 mg/kgBW CBWC | 1 | 245 | 11.8 | 4.82 | 260.83 |
| | | 2 | 264 | 11.8 | 4.47 | 339.79 |
| | | 3 | 259 | 11 | 4.25 | 208.33 |
| | | 4 | 270 | 11.3 | 4.19 | 210.83 |
| | Mean ± SE | | 260 ± 5.33 | 11.5 ± 0.2 | 4.43 ± 0.14 | 254.95 ± 30.76* |

* $P<0.05$ compared with DI control group by Kruskal-Wallis test followed by Mann-Whitney U.

** $P<0.05$ compared with corn oil treated group by Kruskal-Wallis test followed by Mann-Whitney U.

# $P<0.05$ compared with 25 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

## $P<0.05$ compared with 50 mg/kgBW PEITC treated group using by Kruskal-Wallis test followed by Mann-Whitney U.

† $P<0.05$ compared with 714 mg/kgBW FBWC by Kruskal-Wallis test followed by Mann-Whitney U.

## APPENDIX A

Effect of PEITC and Betong watercress on GST activity (μmol/mL/min) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight: Body weight ratio (%) | GST activity (μmol/mL/min) |
|---|---|---|---|---|---|---|
| X | 714 mg/kgBW CBWC | 1 | 259 | 11.8 | 4.56 | 416.67 |
| | | 2 | 247 | 10.8 | 4.37 | 341.46 |
| | | 3 | 255 | 10.8 | 4.24 | 235.83 |
| | | 4 | 266 | 10.7 | 4.02 | 261.67 |
| | Mean ± SE | | 257 ± 3.97 | 11.0 ± 0.26 | 4.3 ± 0.11 | 313.91 ± 40.97 * |
| XI | 1428 mg/kgBW CBWC | 1 | 254 | 10.2 | 4.02 | 339.91 |
| | | 2 | 255 | 10.4 | 4.08 | 386.48 |
| | | 3 | 262 | 11.6 | 4.43 | 241.04 |
| | | 4 | 257 | 11.5 | 4.47 | 214.79 |
| | Mean ± SE | | 257 ± 1.78 | 10.9 ± 0.36 | 4.25 ± 0.12 | 295.56 ± 40.55* |

* $P<0.05$ compared with DI control group by Kruskal-Wallis test followed by Mann-Whitney U.

** $P<0.05$ compared with corn oil treated group by Kruskal-Wallis test followed by Mann-Whitney U.

# $P<0.05$ compared with 25 mg/kgBW PEITC treated group by Kruskal-Wallis test followed by Mann-Whitney U.

## $P<0.05$ compared with 50 mg/kgBW PEITC treated group using by Kruskal-Wallis test followed by Mann-Whitney U.

† $P<0.05$ compared with 714 mg/kgBW FBWC by Kruskal-Wallis test followed by Mann-Whitney U.

## APPENDIX B

Effect of PEITC and Betong watercress on protein content (mg/mL) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight:Body weight ratio (%) | Protein content (mg/mL) |
|---|---|---|---|---|---|---|
| I | Control (distilled water) | 1 | 235 | 8.3 | 3.53 | 6.44 |
| | | 2 | 214 | 9.6 | 4.49 | 7.10 |
| | | 3 | 235 | 9.8 | 4.17 | 7.17 |
| | | 4 | 237 | 8.9 | 3.76 | 7.29 |
| | | 5 | 243 | 10.2 | 4.20 | 6.76 |
| | Mean ± SE | | 232 ± 4.92 | 9.36 ± 0.34 | 4.03 ± 0.17 | 6.95 ± 0.16 |
| II | Corn oil | 1 | 219 | 10 | 4.57 | 4.55 |
| | | 2 | 217 | 8.7 | 4.01 | 3.58 |
| | | 3 | 221 | 9 | 4.07 | 4.59 |
| | | 4 | 208 | 8.6 | 4.13 | 5.59 |
| | | 5 | 231 | 9 | 3.90 | 6.43 |
| | Mean ± SE | | 219 ± 3.69 | 9.06 ± 0.25 | 4.14 ± 0.11 | 4.94 ± 0.49* |
| III | 25 mg/kgBW PEITC | 1 | 235 | 9.4 | 4.00 | 5.26 |
| | | 2 | 223 | 9.3 | 4.17 | 5.55 |
| | | 3 | 194 | 8.1 | 4.18 | 6.32 |
| | | 4 | 203 | 7.9 | 3.89 | 4.82 |
| | | 5 | 224 | 8.4 | 3.75 | 4.32 |
| | Mean ± SE | | 215 ± 7.51 | 8.62 ± 0.31 | 4.00 ± 0.08 | 5.25 ± 0.34 |

Value are presented as mean ± SEM

* $P<0.05$ compared with control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

# $P<0.05$ compared with 25mg/kgBW PEITC treated group using ANOVA followed by post hoc LSD.

# APPENDIX B

Effect of PEITC and Betong watercress on protein content (mg/mL) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight:Body weight ratio (%) | Protein content (mg/mL) |
|---|---|---|---|---|---|---|
| IV | 50 mg/kgBW PEITC | 1 | 239 | 11.5 | 4.81 | 6.32 |
| | | 2 | 230 | 8.7 | 3.78 | 7.17 |
| | | 3 | 217 | 8.1 | 3.73 | 6.98 |
| | | 4 | 222 | 9.7 | 4.37 | 4.77 |
| | | 5 | 206 | 7.8 | 3.79 | 5.47 |
| | Mean ± SE | | 222 ± 5.62 | 9.16 ± 0.67 | 4.1 0 ± 21 | 6.14 ± 0.45 |
| V | 100 mg/kgBW PEITC | 1 | 218 | 9.1 | 4.17 | 6.12 |
| | | 2 | 211 | 8.2 | 3.89 | 6.61 |
| | | 3 | 220 | 9.4 | 4.27 | 6.85 |
| | | 4 | 215 | 9.9 | 4.60 | 9.12 |
| | | 5 | 215 | 8.9 | 4.14 | 7.00 |
| | Mean ± SE | | 215 ± 1.53 | 9.10 ± 0.28 | 4.22 ± 0.12 | 7.14 ± 0.52$^{**,\#}$ |
| VI | 357 mg/kgBW FBWC | 1 | 243 | 10.3 | 4.24 | 8.06 |
| | | 2 | 235 | 12.7 | 5.40 | 9.61 |
| | | 3 | 267 | 11.2 | 4.19 | 5.96 |
| | | 4 | 263 | 11.6 | 4.41 | 5.26 |
| | | 5 | 270 | 11.6 | 4.30 | 7.21 |
| | Mean ± SE | | 255 ± 6.98 | 11.48 ± 0.39 | 4.51 ± 0.23 | 7.22 ± 0.77$^{\#}$ |

Value are presented as mean ± SEM

* $P<0.05$ compared with control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

\# $P<0.05$ compared with 25mg/kgBW PEITC treated group using ANOVA followed by post hoc LSD.

**APPENDIX B**

Effect of PEITC and Betong watercress on protein content (mg/mL) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight:Body weight ratio (%) | Protein content (mg/mL) |
|---|---|---|---|---|---|---|
| VII | 714 mg/kgBW FBWC | 1 | 218 | 9.1 | 4.17 | 7.32 |
| | | 2 | 211 | 8.2 | 3.89 | 6.11 |
| | | 3 | 220 | 9.4 | 4.27 | 6.18 |
| | | 4 | 215 | 9.9 | 4.60 | 5.80 |
| | | 5 | 215 | 8.9 | 4.14 | 6.49 |
| | Mean ± SE | | 215 ± 1.53 | 9.10 ± 0.28 | 4.22 ± 0.12 | 6.38 ± 0.26 |
| VIII | 1428 mg/BW FBWC | 1 | 247 | 11.9 | 4.82 | 5.60 |
| | | 2 | 244 | 10.8 | 4.43 | 6.53 |
| | | 3 | 274 | 12.8 | 4.67 | 7.73 |
| | | 4 | 258 | 11.5 | 4.46 | 7.58 |
| | | 5 | 265 | 12 | 4.53 | 7.10 |
| | Mean ± SE | | 257 ± 5.57 | 11.80 ± 0.33 | 4.58 ± 0.07 | 6.91 ± 0.39# |
| IX | 357 mg/kgBW CBWC | 1 | 245 | 11.8 | 4.82 | 4.97 |
| | | 2 | 264 | 11.8 | 4.47 | 6.76 |
| | | 3 | 259 | 11 | 4.25 | 5.80 |
| | | 4 | 270 | 11.3 | 4.19 | 5.29 |
| | | 5 | 277 | 13.3 | 4.80 | 7.78 |
| | Mean ± SE | | 263 ± 5.41 | 11.84 ± 0.40 | 4.50 ± 0.13 | 6.12 ± 0.51 |

Value are presented as mean ± SEM

* *P*<0.05 compared with control group using ANOVA followed by post hoc LSD.

** *P*<0.05 compared with corn oil treated group using ANOVA followed by post hoc LSD.

\# *P*<0.05 compared with 25mg/kgBW PEITC treated group using ANOVA followed by post hoc LSD.

## APPENDIX B

Effect of PEITC and Betong watercress on protein content (mg/mL) in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight:Body weight ratio (%) | Protein content (mg/mL) |
|---|---|---|---|---|---|---|
| X | 714 mg/kgBW CBWC | 1 | 259 | 11.8 | 4.56 | 6.80 |
| | | 2 | 247 | 10.8 | 4.37 | 8.01 |
| | | 3 | 255 | 10.8 | 4.24 | 6.31 |
| | | 4 | 266 | 10.7 | 4.02 | 7.41 |
| | | 5 | 260 | 10.6 | 4.08 | 7.55 |
| | Mean ± SE | | 257.4 ± 3.14 | 10.94 ± 0.22 | 4.25 ± 0.10 | 6.28 ± 1.18# |
| XI | 1428 mg/kg CBWC | 1 | 254 | 10.2 | 4.02 | 7.14 |
| | | 2 | 255 | 10.4 | 4.08 | 8.03 |
| | | 3 | 262 | 11.6 | 4.43 | 5.91 |
| | | 4 | 257 | 11.5 | 4.47 | 6.44 |
| | | 5 | 266 | 10.7 | 4.02 | 7.48 |
| | Mean ± SE | | 259 ± 2.27 | 10.88 ± 0.29 | 4.20 ± 0.10 | 7.00 ± 0.37# |

Value are presented as mean ± SEM

* $P<0.05$ compared with control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

# $P<0.05$ compared with 25mg/kgBW PEITC treated group using ANOVA followed by post hoc LSD.

## APPENDIX C

Effect of PEITC and Betong watercress on glutathione in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight : Body weight (%) | Total glutathione (mM) | Reduced glutathione (GSH) (mM) | Oxidized glutathione (GSSG) (mM) | GSH:GSSG |
|---|---|---|---|---|---|---|---|---|---|
| I | Control (distilled water) | 1 | 235 | 8.3 | 3.532 | 1.15 | 0.51 | 0.63 | 0.81 |
| | | 2 | 214 | 9.6 | 4.486 | 0.86 | 0.37 | 0.49 | 0.76 |
| | | 3 | 235 | 9.8 | 4.170 | 1.13 | 0.67 | 0.46 | 1.46 |
| | | 4 | 237 | 8.9 | 3.755 | 1.68 | 1.30 | 0.38 | 3.42 |
| | Mean ± SE | | 230 ± 6.06 | 9.15 ± 0.41 | 3.99 ± 0.24 | 1.2 ± 0.17 | 0.71 ± 0.21 | 0.49 ± 0.05 | 1.61 ± 0.63 |
| II | Corn oil | 1 | 219 | 10 | 4.566 | 1.15 | 0.61 | 0.53 | 1.15 |
| | | 2 | 217 | 8.7 | 4.009 | 0.95 | 0.47 | 0.48 | 0.98 |
| | | 3 | 221 | 9 | 4.072 | 1.32 | 0.98 | 0.34 | 2.88 |
| | | 4 | 208 | 8.6 | 4.135 | 1.30 | 0.72 | 0.58 | 1.24 |
| | | 5 | 231 | 9 | 3.896 | 1.39 | 0.85 | 0.54 | 1.5 |
| | Mean ± SE | | 219 ± 3.69 | 9.1 ± 0.25 | 4.14 ± 0.11 | 1.22 ± 0.08 | 0.73 ± 0.09 | 0.49 ± 0.04 | 1.55 ± 0.35 |

Value are presented as mean±SEM

* $P<0.05$ compared with DI control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

### $P<0.05$ compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† $P<0.05$ compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.

## APPENDIX C

Effect of PEITC and Betong watercress on glutathione in liver at 3 days.

| **Group** | **Treatment** | **n** | **Body weight (g)** | **Liver weight (g)** | **Liver weight : Body weight (%)** | **Total glutathione (mM)** | **Reduced glutathione (GSH) (mM)** | **Oxidized glutathione (GSSG) (mM)** | **GSH:GSSG** |
|---|---|---|---|---|---|---|---|---|---|
| III | 25 mg/kgBW PEITC | 1 | 235 | 9.4 | 4.000 | 1.10 | 0.82 | 0.28 | 2.93 |
| | | 2 | 223 | 9.3 | 4.170 | 1.05 | 0.75 | 0.29 | 2.59 |
| | | 3 | 194 | 8.1 | 4.175 | 0.80 | 0.67 | 0.13 | 5.153 |
| | | 4 | 203 | 7.9 | 3.892 | 0.29 | 0.21 | 0.08 | 2.625 |
| | | 5 | 224 | 8.4 | 3.750 | 0.67 | 0.43 | 0.23 | 1.869 |
| | Mean ± SE | | 216 ± 7.51 | 8.6 ± 0.31 | 4.00 ± 0.08 | 0.78 ± 0.15 | 0.58 ± 0.11 | 0.2 ± 0.04**,### | 3.03 ± 0.55 |
| IV | 50 mg/kgBW PEITC | 1 | 239 | 11.5 | 4.812 | 1.08 | 0.66 | 0.42 | 1.57 |
| | | 2 | 230 | 8.7 | 3.783 | 1.39 | 1.06 | 0.32 | 3.31 |
| | | 3 | 217 | 8.1 | 3.733 | 0.91 | 0.54 | 0.37 | 1.46 |
| | | 4 | 222 | 9.7 | 4.369 | 2.34 | 1.89 | 0.45 | 4.2 |
| | | 5 | 206 | 7.8 | 3.786 | 0.36 | 0.30 | 0.06 | 5.00 |
| | Mean ± SE | | 223 ± 5.62 | 9.2 ± 0.67 | 4.10 ± 0.21 | 1.22 ± 0.33 | 0.89 ± 0.28 | 0.33 ± 0.07** | 3.10 ± 0.70 |

Value are presented as mean±SEM

* $P$<0.05 compared with DI control group using ANOVA followed by post hoc LSD.

** $P$<0.05 compared with corn oil treated group using ANOVA followed by post hoc LSD.

### $P$<0.05 compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† $P$<0.05 compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.

## APPENDIX C

Effect of PEITC and Betong watercress on glutathione in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight : Body weight (%) | Total glutathione (mM) | Reduced glutathione (GSH) (mM) | Oxidized glutathione (GSSG) (mM) | GSH:GSSG |
|---|---|---|---|---|---|---|---|---|---|
| V | 100 mg/kgBW PEITC | 1 | 218 | 9.1 | 4.174 | 1.60 | 1.46 | 0.14 | 10.43 |
| | | 2 | 211 | 8.2 | 3.886 | 1.40 | 1.30 | 0.10 | 13 |
| | | 3 | 220 | 9.4 | 4.273 | 0.17 | 0.03 | 0.14 | 0.21 |
| | | 4 | 215 | 9.9 | 4.605 | 0.24 | 0.03 | 0.21 | 0.14 |
| | | 5 | 215 | 8.9 | 4.140 | 0.60 | 0.46 | 0.14 | 3.28 |
| | Mean ± SE | | 216 ± 1.53 | 9.1 ± 0.28 | 4.22 ± 0.12 | 0.8 ± 0.30 | 0.65 ± 0.31 | 0.15 ± 0.02** | 5.41 ± 2.60** |
| VI | 357 mg/kgBW FBWC | 1 | 243 | 10.3 | 4.239 | 1.51 | 1.25 | 0.26 | 4.81 |
| | | 2 | 235 | 12.7 | 5.404 | 2.12 | 1.79 | 0.34 | 5.26 |
| | | 3 | 267 | 11.2 | 4.195 | 1.04 | 0.80 | 0.23 | 3.48 |
| | | 4 | 263 | 11.6 | 4.411 | 0.88 | 0.61 | 0.27 | 2.26 |
| | Mean ± SE | | 252 ± 7.72 | 11.5 ± 0.5 | 4.56 ± 0.28 | 1.39 ± 0.28 | 1.11 ± 0.26 | 0.28 ± 0.02* | 3.95± 0.69 |

Value are presented as mean±SEM

* *P*<0.05 compared with DI control group using ANOVA followed by post hoc LSD.

** *P*<0.05 compared with corn oil treated group using ANOVA followed by post hoc LSD.

### *P*<0.05 compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† *P*<0.05 compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.

## APPENDIX C

Effect of PEITC and Betong watercress on glutathione in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight : Body weight (%) | Total glutathione (mM) | Reduced glutathione (GSH) (mM) | Oxidized glutathione (GSSG) (mM) | GSH:GSSG |
|---|---|---|---|---|---|---|---|---|---|
| VII | 714 mg/kgBW FBWC | 1 | 218 | 9.1 | 4.174 | 1.53 | 1.14 | 0.38 | 3 |
| | | 2 | 211 | 8.2 | 3.886 | 1.19 | 0.97 | 0.22 | 4.41 |
| | | 3 | 220 | 9.4 | 4.273 | 0.67 | 0.06 | 0.62 | 0.097 |
| | | 4 | 215 | 9.9 | 4.605 | 1.08 | 0.72 | 0.36 | 2 |
| | | 5 | 215 | 8.9 | 4.140 | 0.84 | 0.63 | 0.21 | 3 |
| | Mean ± SE | | 216 ± 1.53 | 9.1 ± 0.28 | 4.22 ± 0.12 | 1.06 ± 0.15 | 0.7 ± 0.19 | 0.36 ± 0.07 | 2.50 ± 0.71 |
| VIII | 14287 mg/kg FBWC | 1 | 247 | 11.9 | 4.818 | 2.69 | 2.47 | 0.22 | 11.23 |
| | | 2 | 244 | 10.8 | 4.426 | 1.73 | 1.55 | 0.19 | 8.16 |
| | | 3 | 274 | 12.8 | 4.672 | 0.95 | 0.72 | 0.23 | 3.13 |
| | | 4 | 258 | 11.5 | 4.457 | 1.48 | 1.17 | 0.31 | 3.77 |
| | Mean ± SE | | 256 ± 6.79 | 11.8 ± 0.42 | 4.59 ± 0.09 | 1.71 ± 0.36* | 1.48 ± 0.37*,† | 0.24 ± 0.03* | 6.57 ± 1.97*,† |

Value are presented as mean±SEM

* $P<0.05$ compared with DI control group using ANOVA followed by post hoc LSD.

** $P<0.05$ compared with corn oil treated group using ANOVA followed by post hoc LSD.

### $P<0.05$ compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† $P<0.05$ compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.

## APPENDIX C

Effect of PEITC and Betong watercress on glutathione in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight : Body weight (%) | Total glutathione (mM) | Reduced glutathione (GSH) (mM) | Oxidized glutathione (GSSG) (mM) | GSH:GSSG |
|---|---|---|---|---|---|---|---|---|---|
| IX | 357 mg/kgBW CBWC | 1 | 245 | 11.8 | 4.816 | 1.70 | 1.45 | 0.25 | 5.80 |
| | | 2 | 264 | 11.8 | 4.470 | 3.23 | 2.92 | 0.31 | 9.42 |
| | | 3 | 259 | 11 | 4.247 | 1.26 | 1.06 | 0.20 | 5.3 |
| | | 4 | 270 | 11.3 | 4.185 | 1.35 | 0.97 | 0.38 | 2.55 |
| | Mean ± SE | | 260 ± 5.33 | 11.5 ± 0.2 | 4.43 ± 0.14 | 1.89 ± 0.46*,† | 1.6 ± 0.45*,† | 0.29 ± 0.44* | 5.76 ± 1.38* |
| X | 714 mg/kgBW CBWC | 1 | 259 | 11.8 | 4.556 | 1.08 | 0.76 | 0.32 | 2.38 |
| | | 2 | 247 | 10.8 | 4.372 | 1.80 | 1.50 | 0.30 | 5 |
| | | 3 | 255 | 10.8 | 4.235 | 1.15 | 0.65 | 0.50 | 1.3 |
| | | 4 | 266 | 10.7 | 4.023 | 1.40 | 1.06 | 0.35 | 3.03 |
| | Mean ± SE | | 257 ± 3.97 | 11.0 ± 0.26 | 4.3 ± 0.11 | 1.36 ± 0.16 | 0.99 ± 0.19 | 0.37 ± 0.04 | 2.93 ± 0.76 |

Value are presented as mean±SEM

* *P*<0.05 compared with DI control group using ANOVA followed by post hoc LSD.

** *P*<0.05 compared with corn oil treated group using ANOVA followed by post hoc LSD.

### *P*<0.05 compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† *P*<0.05 compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.

## APPENDIX C

Effect of PEITC and Betong watercress on glutathione in liver at 3 days.

| Group | Treatment | n | Body weight (g) | Liver weight (g) | Liver weight : Body weight (%) | Total glutathione (mM) | Reduced glutathione (GSH) (mM) | Oxidized glutathione (GSSG) (mM) | GSH:GSSG |
|---|---|---|---|---|---|---|---|---|---|
| XI | 1428 mg/kgBW CBWC | 1 | 254 | 10.2 | 4.016 | 1.55 | 1.26 | 0.29 | 4.2573 |
| | | 2 | 255 | 10.4 | 4.078 | 1.81 | 1.57 | 0.24 | 6.5710 |
| | | 3 | 262 | 11.6 | 4.427 | 1.44 | 0.96 | 0.49 | 1.9538 |
| | | 4 | 257 | 11.5 | 4.475 | 1.18 | 0.84 | 0.35 | 2.4267 |
| | Mean ± SE | | 257 ± 1.78 | 10.9 ± 0.36 | 4.25 ± 0.12 | 1.5 ± 0.13 | 1.15 ± 0.16 | 0.34 ± 0.05 | 3.80 ± 1.05 |

Value are presented as mean±SEM

* *P*<0.05 compared with DI control group using ANOVA followed by post hoc LSD.

** *P*<0.05 compared with corn oil treated group using ANOVA followed by post hoc LSD.

### *P*<0.05 compared with 100 mg/kg BW PEITC using ANOVA followed by post LSD.

† *P*<0.05 compared with 714 mg/kg BW FBWC using ANOVA followed by post hoc LSD.