CHAPTER I

INTRODUCTION

Cadmium

Cadmium is a group IIB metal that has an atomic weight of 112.41 g/mol and it exists in the 0 or 2^+ oxidation states. Pure cadmium is a soft, silver-white metal, not usually present in the environment in its pure form but as a mineral compound with other elements such as oxygen (cadmium oxide; CdO), chloride (cadmium chloride; $CdCl_2$) or sulfur (cadmium sulfate; $CdSO_4$). It is extracted during the production of other metals such as zinc, lead and copper and used in industrial and household products, mainly batteries, pigments, metal coatings, plastics and some metal alloys (ATSDR, 1998).

Toxicokinetics of cadmium

Absorption

Human are generally exposed to cadmium by two main routes, inhalation and ingestion. Absorption of cadmium through the skin is relatively insignificant although small amounts of cadmium can be absorbed percutaneously over a long period of exposure (Wester *et al*., 1992). The principal compound for oral exposure is cadmium chloride as it is highly soluble in water. By contrast, cadmium oxide is mainly inhaled. Approximately 60% of inhaled cadmium is translocated to the

gastrointestinal tract in rats (Moore *et al*., 1973). Pulmonary absorption of cadmium is more efficient than absorption via the gastrointestinal tract which is only about 1-2% efficient in mice and rats, 0.5-3% in monkeys, 2% in goats and 5% in pigs and lambs. Cattle (at 16%) seem to be most efficient mammals for gastrointestinal absorption of cadmium (Zalups and Ahmad, 2003).

Distribution and metabolism

After absorption, cadmium is primarily bound to albumin in blood serum (Figure 1). This is the dominant form of cadmium in plasma shortly after uptake before transport to the various body pools. The liver is the primary uptake and accumulation site. Cadmium ions released from the liver induce the synthesis of metallothionein (MT) which binds the cadmium ions (Cd) forming a nontoxic complex (CdMT). CdMT is distributed throughout the body especially in the kidney. Because of its small molecule size, the complex is filtered by the glomerulus membrane and efficiently taken up by renal proximal tubular cells (Nordberg, 1984; Fowler, 1992; Jarup *et al*., 1998).

After entering renal tubular cells via pinocytosis, the CdMT compound is catabolized by lysosomes to release cadmium ions. Thus, any cadmium in the kidney which is bound to MT is the result of *de novo* synthesis (Figure 2) (Kido *et al*., 2003). This process may account for the long biological half-life of cadmium in the kidney where the element may be retained 10-20 years.

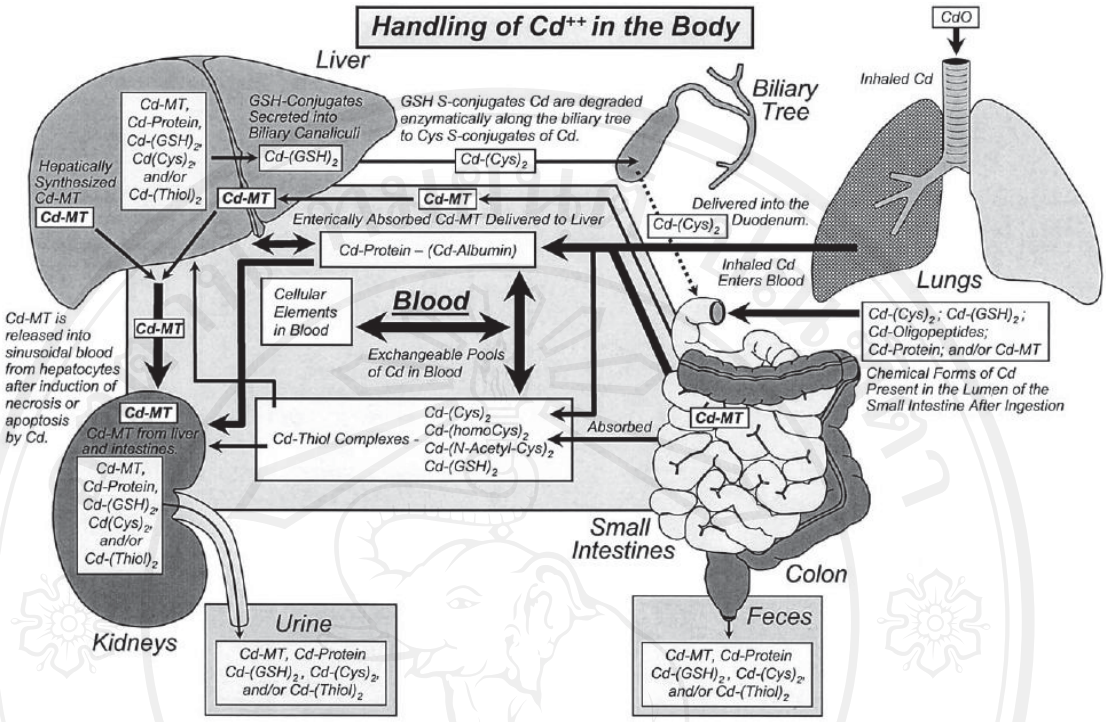

Figure 1 Toxicokinetic diagram of cadmium (Zalups and Ahmad, 2003)

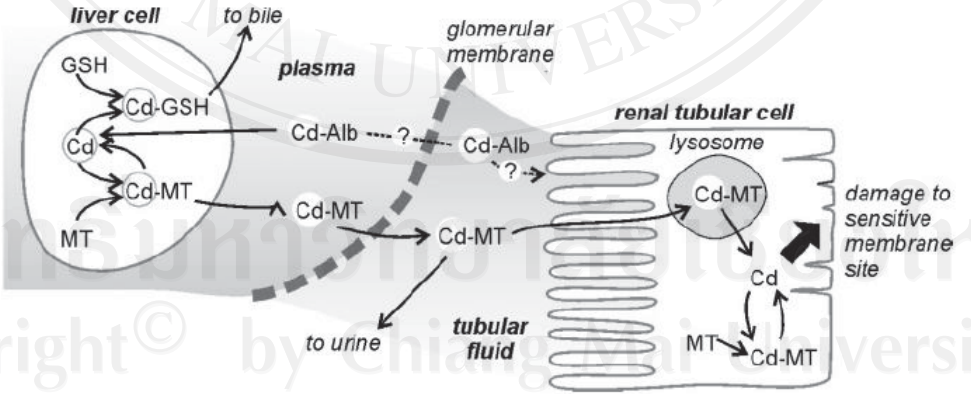

Figure 2 Pathways of cadmium uptake and interaction with target sites in the kidney (Kido *et al*., 2003)

Cadmium toxicity occurs when metallothionein synthesis is insufficient to match the demand and free cadmium ions overwhelm other defense mechanisms as well and causes toxicities (Nordberg, 1984; Lauwerys and Bernard, 1986; WHO, 1992; Jarup *et al*., 1998).

Excretion

Because cadmium is eliminated very slowly by urinary and fecal mechanisms (0.01-0.02% of the body burden per day), it has a long biological half-life. Urinary excretion of cadmium increases when cadmium-induced renal tubular damage occurs and is associated with age increasing body burden. The majority of fecal cadmium is unabsorbed metal passing through the alimentary tract. Cadmium is also eliminated through hair and breast milk but these routes are of limited importance for total excretion and do not significantly alter the biological half-life (ATSDR, 1998).

Toxicities of cadmium

The main routes of acute cadmium toxicity are by ingestion or inhalation. Ingestion of excessive dietary cadmium (more than 15 mg cadmium/kg) causes vomiting and diarrhea. Inhalation of high concentrations of cadmium (above 5 mg/m^3) causes pneumonitis and may be lethal (Kido *et al*., 2003).

Chronic toxicity of cadmium mainly causes nephrotoxicity and hepatotoxicity. Nephrotoxicity affects calcium metabolism and causes osteopathy. Chronic toxicity of cadmium also affects the lungs, reproductive system, cardiovascular system, immune and hematopoietic system, nervous system and is carcinogenic.

Nephrotoxicity: The proximal tubule has long been recognized as a major target in cadmium-induced nephropathy with proximal tubular necrosis and interstitial nephritis as prominent features (Aughey *et al*., 1984; Asar *et al*., 2004). However, renal glomeruli are also exposed to circulating metals during plasma filtration and may also be targets of cadmium (Templeton and Liu, 2010). The earliest manifestations of tubular toxicity are increased excretion of low-molecular weight proteins such as β2-microglobulin, α1-microglobulin and retinol-binding protein. Increased urinary excretion of markers of cytolysis have been reported such as the lysosomal enzyme N-acetyl glucosaminidase (Teeyakasem *et al*., 2007; Ferguson *et al*., 2008; Gonick, 2008; Prozialeck and Edwards, 2010) and the kidney injury molecule-1, a recently discovered biomarker of early stage of cadmium induced proximal tubule injury (Vaidya *et al*., 2006; Prozialeck *et al*., 2007; Prozialeck *et al*., 2009; Panyamoon *et al*., 2009).

Hepatotoxicity: The liver is a major storage and metabolic organ for the *in vivo* handling of cadmium. It has a large reserve capacity and ability to induce anti-oxidant systems. Cadmium induces oxidative stress in mediating cadmium liver toxicity and finites nature of protective antioxidant systems in attenuating cell damage (Fowler, 2009).

Osteopathy: According to renal dysfunction, vitamin D which is important for bone metabolism cannot metabolize to an active form. This affects bone metabolism and causes osteomalacia or osteoporosis. Long-term exposure to high doses of cadmium causes Itai-itai disease. This disease affects mainly women and is characterized by severely impaired tubular and glomerular function, generalized

osteomalacia and osteoporosis with resultant multiple bone fracture (Satarug *et al*., 2010).

Lung: Cadmium-induced oxidative stress is a major consequence of mediating cadmium toxicity in the lung in relation to both asthma (Willer *et al*., 2005) and pulmonary fibrosis (Kirschvink *et al*., 2006).

Reproductive system: Cadmium affects both male and female reproductive systems. Cadmium causes disruption of the vascular system in the testis, blood-testis barrier and acute testicular damage (Prozialeck *et al*., 2008; Sue *et al*., 2009). Cadmium also accumulates in the ovary and has been associated with oocyte and embryo development (Thompson and Bannigan, 2008).

Cardiovascular system: Cadmium effects the blood vasculature leading to both hypertensive effects and morphological damage of the capillary system. It may also interact with central cellular signaling systems (Misra *et al*., 2003; Rockwell *et al*., 2007). The mechanisms of cadmium toxicity involve the cellular signaling systems and oxidative stress (Fowler, 2009).

Immune and hematopoietic systems: Cadmium exposure alters immune cell functions both in terms of protein expression patterns and of normal immune cell function. Cadmium alters phagocytosis in immune cells (Di Gioacchino *et al*., 2008), cell and humoral immunity (Dan *et al*., 2000; Pathak and Khandelwal, 2006) and regulation of cadmium responsive genes (Dakeshita *et al*., 2009).

Chronic oral cadmium exposure in rats leads to hypochronic anemia (Ktapcinska *et al*., 2000) through the direct impairment of erythropoietin-producing tubular cells (Horiguchi *et al*., 2006).

Neurotoxicity: Cadmium induces neurotoxicity including neurological disturbances and changes in the normal neurochemistry of the brain. The mechanisms of neurotoxicity involve the role of the blood-brain barrier, oxidative stress, interference with calcium and zinc dependent processes and apoptosis induction as well as the modulatory effect of metallothionein (Mendez-Armenta and Rios, 2007).

Carcinogenicity: The International Agency for Research on Cancer classifies cadmium as a human carcinogen (group I) on the basis of evidence of carcinogenicity in humans and experimental animals. The European Commission has classified some cadmium compounds as possibly carcinogenic (Carcinogen Category 2). Exposure to cadmium has been associated with cancer of lung, prostate, kidney and breast (Waalkes, 2003). The mechanisms of cadmium induced cancer involve aberrant gene expression, inhibition of DNA damage repair, induction of oxidative stress and inhibition of apoptosis (Joseph, 2009; Templeton and Liu, 2010).

Cadmium contamination in Thailand

In Mae Sot District, Tak Province, Thailand, paddy fields irrigated from two creeks (Mae Tao and Mae Ku) were found to contain markedly elevated cadmium levels in 2001-2004 (Simmon *et al*., 2005; Swaddiwudhipong *et al*., 2007). Both creeks drain a zinc rich area where a zinc mine has operated for more than 20 years. Soil cadmium concentrations in Mae Sot District ranged from 0.5 to 284 mg/kg. The

highest levels were 1,800 times the Thai Investigation Level for cadmium in soils. Cadmium concentrations in rice grain ranged from 0.05 to 7.7 mg/kg and over 90% of the rice grain samples contained cadmium concentrations exceeding the Codex Committee on Food Additives and Contaminants draft Maximum Permissible Level for rice grain of 0.2 mg cadmium/kg (Simmon *et al*., 2005). Persons surveyed who mainly consumed rice grown in the contaminated areas had higher urinary cadmium than those who did not (Swaddiwudhipong *et al*., 2007) and those who were farmers had higher risk of renal dysfunction (Teeyakasem *et al*., 2007).

Urinary cadmium levels were associated with urinary levels of β2-microglobulin, N-acetyl-β-D-glucosaminidase, total protein and kidney injury molecule-1, all indicators of renal tubular dysfunction and renal damage (Limpatanachote *et al*., 2009; Panyamoon *et al*., 2009; Honda *et al*., 2010). Chronic dietary cadmium exposure in people from Mae Sot impaired calcium reabsorption in renal tubules and accelerated bone resorption (Nambunmee *et al*., 2010).

Recently report by Swaddiwudhipong *et al* (2010) revealed increased urinary cadmium concentrations in cadmium exposed populations from 12 villages in Tak Province were associated with an increased prevalence of hypertension in men and women. The study demonstrated a positive correlation between urinary cadmium and systolic blood pressure but not with diastolic blood pressure.

Chronic cadmium toxicity amongst Mae Sot inhabitants is a public concern. The production of rice and other food crops in the contaminated area should be controlled to prevent bioaccumulation of cadmium in humans.

Thai traditional medicine uses Rang Chuet (*Thunbergia laurifolia* Lindl.) as an antidote to several poisons. It is usually consumed as Rang Chuet tea.

***Thunbergia laurifolia* Lindl.**

Thunbergia laurifolia Lindl. (Figure 3 and 4) is commonly known in Thai as Rang Chuet (Babbler's Bill in English). The plant belongs to the botanical family of Acanthaceae (Thongsaard and Marsden, 2005) and is a large strong woody climber with green stem segment. The leaves are simple, opposite, green and smooth, ovate or oblong in shape, 4-5 cm wide, 8-10 cm long and slightly lobed. *T. laurifolia* can be divided into three types by flower color: white, yellow or purple. The purple varieties are believed to contain compounds that deliver health benefits particularly from material of the stem, root and leaves. The trumpet-shaped flower has 5 petals with no fragrance, 3-4 flowers per bunch and pendulous racemes. The flower in full bloom is 7-8 cm in diameter. The center of the flower is white in color with four stamens. The bract is 2.5 cm in length and green color with reddish brown blotches. The plants flower from November to January. If the flower is fertilized, a pod shaped fruit forms. The end of the pod is pointed like a bird's bill.

T. laurifolia grows in evergreen forests, grove forests and gullies. The plant mostly confined to Asian regions such as Thailand, India and Malaysia. It can be grown in various types of soil. It breeds is by seeding, plant cutting and scion grafting (Thiangbuntham, 1999; Yaithumsan, 1999).

Figure 3 *T. laurifolia* in Ob Khan National Park, Hang Dong District, Chiang Mai Province

Figure 4 *T. laurifolia* flower

(http://www.biologie.uni-hamburg.de/b-online/vascular/images/thu_lau_cu.jpg)

Traditional uses of *T. laurifolia*

Thai traditional herbs textbook describe the herb's properties as follows:

Leaves (fresh) : antipyretic, insecticide and toxin inhibition (Wuttithamwat, 1997; Thiangbunthum, 1999)

Leaves (dried) : antidote for several toxicants such as arsenic, strychnine, ethanol and insect toxin (Chanawirat, 2000)

Stem (dried) : antipyretic effect (Panichayupakaranunt, 2011)

Root (dried) : anti-inflammatory, heals swelling, antipyretic effect (Wasawut, 1976)

Traditional recipes using *T. laurifolia* as an antidote for toxicants:

1. Approximately 7-10 fresh leaves are pounded in water or rice-wash water. They are squeezed before administration as a poisoning treatment (Utokpach, 1976).

2. About two handfuls of leaves and vine are ground in water and then boiled for ten minutes. It is suggested that drinking the solution as often as needed reduces the effect of poisons on the body (Panichayupakaranunt, 2011).

3. The root or 7-10 fresh leaves are ground in rice-wash water. This remedies the toxic effects of insecticides and other poisonous substances such as alcohol, toxic mushroom or toxic vegetables (Tejasen, 1978).

Chemical constituents of *T. laurifolia*

Jitpuwngam (1979) reported the constituents of *T. laurifolia* leaves extracted using water and methanol consisted of methionine, glycine, serine and unidentified amino acid. Eight steroids and a carotenoid were detected in the petroleum ether extract.

Krairatcharoen *et al* (1999) studied active constituents of *T. laurifolia* leaves by phytochemical screening, chemical tests and thin layer chromatography. Six compounds were identified which gave violet spot with vanillin phosphoric acid spray reagent; four compounds were in the ethanol and two compounds in the hexane extract. UV spectroscopic showed that the pure compounds had the same basic structure as Stigmasterol.

Supalaknaree and Krungkrai (1999) extracted and purified apigenin from *T. laurifolia* by organic solvent extraction and high performance liquid chromatography. Apigenin (Figure 5) is a plant flavonoid which possesses many pharmacological activities. The yields of apigenin were 0.03%, 0.004% and 0.013% from water, methanol and ethanol extracts, respectively.

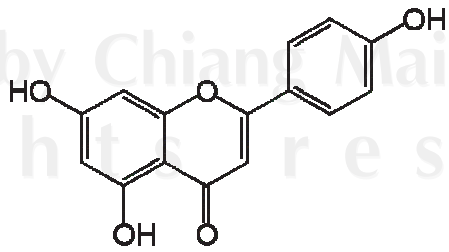

Figure 5 Apigenin

(http://upload.wikimedia.org/wikipedia/commons/b/bf/Apigenin.png)

Kanchanapoom *et al*. (2002) reported two iridoid glucosides; 8-epi-grandifloric acid (Figure 6) and 3'-*O*-β-glucopyranosyl-stilbericoside (Figure 7) isolated from the aerial part of *T. laurifolia* along with seven known compounds; benzyl β-glucopyranoside, benzyl β-(2'-*O*-β-glucopyranosyl) glucopyranoside, grandifloric acid, (*E*)-2-hexenyl β-glucopyranoside, hexanol-β-glucopyranoside, 6-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosylapigenin.

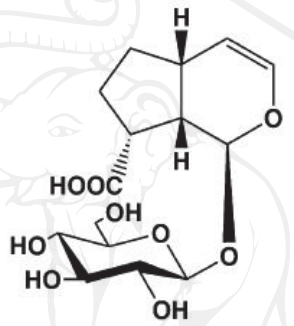

Figure 6 8-epi-grandifloric acid

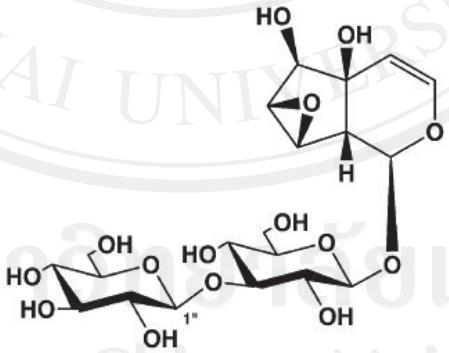

Figure 7 3'-*O*-β-glucopyranosyl-stilbericoside

The flowers of *T. laurifolia* are also reported to contain delphinidin 3:5-di-o-β-D-glucopyranoside, apigenin and apigenin-7-o-β-D-glucopyranoside (Purnima and Gupta, 1978).

Pharmacology of *T. laurifolia*

Chamreondararassame (1978) tested the antipyretic property of *T. laurifolia* in rats by intraperitoneal injection (15g/kg BW). The study concluded that the *T. laurifolia* leaf extract decreased body temperature by modifying the temperature control center in the brain and/or induced vasodilatation to reduce heat in the body.

T. laurifolia leaf extracts antagonized the toxic effects of Folidol E-605 in rats. Administration of *T. laurifolia* leaf extract decreased mortality rate in rats from 56.67 ± 3.33 to 5.00 ± 2.87% by either the intraperitoneal or intrasubcutaneous injection (Ruangyuttikarn, 1980).

Wisitpongpan (2003) reported that aqueous extracts of *T. laurifolia* leaves at a single oral dose (10g/kg BW) did not alter a general behavior and a feature of the visceral organs in rats. The extract at an oral dose (500 mg/kg BW) given 28 days continually did not produce toxic effects or changes in gross morphology of the internal organs of the rats, did not affect behavioral patterns, did not induce free radical formation and was not a bacterial mutagen.

T. laurifolia disrupted drug-seeking behavior by antagonism of dopamine D_3 receptors in a manner similar to 1-(4-(2-Naphthoylamino)-butyl)-4-(2-methoxyphenyl)-1A-piperazine HCl which has recently been reported to have potential in the treatment of addiction and withdrawal (Thongsaard and Marsden, 2002). Moreover, *T. laurifolia* increased neuronal activity in specific brain regions responsible for reward and locomotory behavior (Thongsaard and Marsden, 2005).

T. laurifolia leaf extract had no effect on the induction of micronucleus formation in human lymphocytes or rat bone marrow cells. It was an effective antimutagenic substance for methomyl induced genotoxicity (Boonyarat, 2004). It also has hypoglycemic properties since treatment of alloxan-induced diabetic rats decreased levels of blood glucose and recovered some beta-cells. Furthermore, the extract of *T. laurifolia* did not exert an improvement of reproductive system which was deteriorated by diabetes (Aritajat *et al*., 2004).

T. laurifolia could also be a protective agent for methomyl-induced cholinesterase inhibition. An extract increased the number of acetylcholinesterase-positive neurons and the color intensity of nerve fibers in rat's duodenum myenteric plexuses after reducing both numbers and color intensity of the neurons and nerve fibers induced by methomyl insecticide (Chaiyasing, 2005). *T. laurifolia* showed hepatoprotective activity against ethanol induced liver injury in both primary cultures of rat hepatocytes and rat. Treatment with *T. laurifolia* extract increased the viability of primary cultures of rat hepatocytes, decreased the release of transaminase and promoted rat liver recovery after treatment with ethanol (Pramyothin *et al*., 2005).

A recent study of *T. laurifolia* to alleviate lead poisoning was reported by Tangpong and Satarug (2010). Co-treatment with the extract alleviated adverse effects of lead on learning deficit and memory loss evaluated by a water maze swimming test, restored caspase-3 activity and maintained total anti-oxidant capacity and anti-oxidant enzymes in the brain.

Detoxification of cadmium

Cadmium toxicity has been reported to be ameliorated by zinc. Zinc consumption is reported to prevent cadmium accumulation in organs (Brzoska *et al*., 2007; Bulat *et al*., 2008). Zinc is reported to protect against cadmium body burden and toxic effects on kidneys, liver and skeleton including histopathological changes (Barbier *et al*., 2005; Jihen *et al*., 2008; Jihen *et al*., 2010). Zinc also plays a protective role in lipid metabolism alterations caused by cadmium (Jemai *et al*., 2007; Jemai *et al*., 2010). Although, zinc is an essential metal, excessive exposure to zinc also causes toxicity (Klaassen, 1995). Therefore, traditional herbs might be a better alternative treatment for prevention of cadmium toxicity.

Onion and garlic are reported to lessen cadmium induced nephrotoxicity, reduce cadmium accumulation in organs (Massadeh *et al*., 2007; Pari *et al*., 2007), inhibit lipid peroxidation and enhance antioxidant system (Zhang *et al*., 2005; Pari and Murugavel, 2007; Suru, 2008).

Ginger fed rats had lower levels of liver enzymes-induced by cadmium (Egwurugwu *et al*., 2007). Experiments showed ginger had both prophylactic and therapeutic cadmium detoxification effects.

Extracts from *Panax ginseng*, a medical plant cultivated in Korea, Japan, China and Russia has constituents reported to exhibit both anti-stress and antioxidant activities and *Spirulina platensis*, a blue-green alga rich in proteins and reputed to be an external source of the vital antioxidant enzyme superoxide dismutase reduced

hepatotoxicity induced by cadmium and minimized histopathological changes. The antioxidant properties may mediate these protective effects (Karadeniz *et al*., 2009).

Picroliv, an active constituent of *Picrorhiza kurroa*, a well-known traditional plant of Indian origin, has proven hepatoprotective potential against several hepatotoxicants administrated to rats. The morphological improvement after Picroliv treatment showed signs of reversibility and was further corroborated by decreased excretion of several urinary proteins and enzymes. The hepatic and renal cadmium levels were also markedly lowered (Yadav and Khandelwal, 2006).

Other substances in vegetables and fruits that have been used to reduce cadmium induced nephrotoxicity and hepatotoxicity are vitamin E (El-Demerdash *et al*., 2004; Nemmiche *et al*., 2007; Kara *et al*., 2008), β-carotene (El-Demerdash *et al*., 2004), quercitin (Vicente-Sanchez *et al*., 2008; Renugadevi and Prabu, 2010a), naringenin (Renugadevi and Prabu, 2009; Renugadevi and Prabu, 2010b).

However, there are no reports of cadmium detoxification by *T. laurifolia* leaf extract. Therefore, the hypothesis of this thesis is that *T. laurifolia* leaf extract can reduce cadmium toxicity.

Objective of the study

Inhabitants in cadmium polluted areas in the Mae Sot District, Tak Province, Thailand have had high cadmium exposure causing high risk of renal dysfunction and liver damage. This health issue has been of public interest and concern since 2004. Methods to reduce health risk were considered and the traditional herb, *T. laurifolia* was selected for investigation as agent for cadmium detoxification.

The objectives of this thesis were

1. to establish a rat model showing renal and hepatic injuries induced by cadmium exposure
2. to detoxify the cadmium using *T. laurifolia* leaf extract and
3. to investigate the chemical constituents of the herb