CHAPTER 1

INTRODUCTION

1.1 Rationale of the study

Cigarette smoke that contains an abundance of free radicals and pro-oxidant species is known to cause a serious health problems worldwide (**Pryor *et al.*, 1993**; **Singh *et al.*, 2008**). More than 5 million people per year currently die from acute and chronic diseases, such as coronary heart disease, chronic obstructive pulmonary disease (COPD) and cancers (**Mackay *et al.*, 2006**) and tendency being increase to over 8 million of death rate per year in 2030 (**WHO 2008**). According to current evaluations, there are 1.1 billion smokers worldwide, especially among young teenagers aged around 15 years old (**Ezzati *et al.*, 2003**).

Thousands of chemicals in tobacco smoke and over 4,000 chemical substances in cigarettes are toxic and carcinogenic agents affecting the human body with free radicals and nicotine (**Church and Pryor, 1985**). In addition, the basic knowledge of free radicals has strongly claimed a result of tobacco smoke that is a powerful risk factor of degeneration, mutation, aging and several serious diseases (**Jonathan, 2007**). Nicotine in cigarette smoke directly contributes the catecholamine neurotransmitters releasing in human brain, especially dopamine that is a precursor of both adrenaline and noradrenaline neurotransmitters (**Benowitz *et al.*, 2002**; **Kappor and Jones, 2005**). Previous study reported that the nicotine level in human plasma after cigarette smoking was classified into high, medium and low yields with the range from low to high concentration (95.6 and 236.7 nmol/L) even though after morning and afternoon smoking (**Russell *et al.*, 1975**). From some adverse effects of nicotine on the oxidative stress status and chromosome aberration were reported (**Mundel and David, 2006**) and it has been proposed as an inflammatory inducer by activating the nuclear factor kappa-B (NF-κB) and apoptosis pathway within the cells (**Crowley-Webere *et al.*,**

2003). But, these result finding are still controversial and no any report confirmed on the toxicity or side effects from directly nicotine administrated in active smoker (**Kirkham *et al.*, 2004**; **Bloomer *et al.*, 2008**; **Alberg, 2002**).

Dopamine is an important neurotransmitter that affects to the heart rate and blood pressure, as well as behavior and cognition, sexual performance, mood, learning capacity, sleep, memory, motivation, lactation and involuntary movement (**Benowitz *et al.*, 2002**). Some evidences documented that nicotine could enhance motivation during prolonged exercise (**O'Neill *et al.*, 1991**), improved exercise endurance (**Mundel and David, 2006**) and induced oxidative stress condition (**Wongwiwatthananukit, 2003**). Interestingly, previous evidence showed that adverse effects of nicotine involved the inflammatory status and oxidative stress (**Crowley-Weber *et al.*, 2003**) by decreasing on glutathione (GSH) and increasing on malondialdehyde (MDA) (**Yildiz *et al.*, 1999**). Therefore, smoking is a major health problem and main risk factor for lung cancer, chronic lung ailments and many diseases around the world that must be resolved.

Nowadays, Thai Government has been campaigned and widely promotes for a long time, by introducing various choices, such as behavioral counseling with or without pharmacotherapy at a national call phone 1,600 service or setting the smoking cessation clinic at every hospitals. Previous evidences showed that behavioral combined with pharmacotherapy had the successful long-term abstinence rate of approximately 30% **(Wongwiwatthanukit, 2003; Fiore *et al.*, 2008; Stead *et al.*, 2008**). Nicotine replacement therapy (NRT) is the best medicinal choice in smoking cessation protocol in case of heavy smokers and increases the chance of smoking cessation by 50 to70% compared to a placebo or non treatment (**Prochazka, 2000**). But the major disadvantages of this approach are high cost and unwanted side effects such as nausea, dry mouth, weight gain and sedation (**Chea *et al.*, 2006**). Because of the low economic status in Thai people who want to use these NRT drugs, therefore, this is also possibly the main problem on unsuccessful stop smoking.

Presently, traditional Thai herbs are challenging choices for the purpose of stop smoking. Peep (*Millingtonia hortensis* Linn.), Shallot (*Allium ascalonicim* Linn.), Snow

lotus (*Saussurea laniceps.*) and Mor Noi (*Vernonia cinerea* Less.) can be used for stop smoking. From all plants, *Vernonia cinerea* Less. (VC) was only clinically applied and studied in Thailand **(Wongwiwatthanukit, 2003; Leelarungrayub *et al.*, 2010).**

Vernonia cinerea Less. (VC) is classified into the *Vernonia* (*Asteraceae*) family (**Toyang and Verpoorte, 2013**) that is the largest genus close to 1,000 species (**Keely and Jones, 1979**). There are many genus which is being interested around the world for many medicinal benefits. This plant is natural grown in many countries in Asia as same as in the Northern part of Thailand as Chiang Mai province.

In the previous records about the traditional medicine plants have been suggested that VC is widely used as a traditional medicine in Thailand and other countries for relieving cigarette craving, asthma, cough, fever, malaria, arthritis and urinary calculi (**Misra *et al.*, 1993; Latha *et al.*, 1998; Iwalewa *et al.*, 2003; Mazumder *et al.*, 2003**), including the cytotoxic effect on cancer cells (**Pratheeshkumar and Kuttan, 2012**). Interested suggestion for smoking cessation, VC has been reported since 1985 (**Lhieochaiphat, 1985**) and recorded in a medicinal plant report in Thailand (**Bunyapraphatsara, 2005**). In previous clinical study at the smoking cessation clinic, Thanyarak Institute, Pathumthani, Thailand by Wongwiwatthananukit and co-works who studied a mixed VC crude material in tea bag for 14 days and results showed the higher non-significant of the continuous abstinence rate (CAR) (28.1%) than in the control group (21.9%) (**Wongwiwatthananukit *et al.*, 2009**). But this study was not evaluate any active compounds. In the other hand, there have some evidences on the flavonoid and terpenoid in VC from ethanolic extraction, as well as steroids, saponin, alkaloids, phenols, tannins and proteins, including N-hexadecanoic acid (42.88%), 1,2-benzene-dicarboxylic acid (23%), squalence (11.31%), caryophyllene oxide (2.31%), guaiol (1.75%), octadecanoic acid (4.41%) and 9,12-octadecanoic (9.38%) that exhibited the antioxidant and anti-inflammatory activities *in vitro* and in rats (**Mishra *et al.*, 1984; Latha *et al.*, 1998; Leelaprakash *et al.*, 2011**). But these evidences are still controversial and cannot conclude any mechanisms on stop smoking in human smokers. Furthermore, the last study of VC condense juice from crude mixed parts combined with exercise in active smokers at Chiang Mai province in Thailand, that presented a higher percentage of people who could stop smoking (59.2% in light

cigarette and 54.47% in self-rolling cigarette types), moreover also reduced the oxidative stress status such as malondiladehyde (MDA) from lipid peroxidation, protein hydroperoxide and improved total antioxidant capacity (TAC) (**Leelarungrayub *et al.*, 2010**). Therefore, these results are very interesting on its antioxidant activity and active compounds relating on stop smoking and acute toxicity VC condense juice from administration that need to be confirmed. Presently, VC plant has been strongly approved for a smoking cessation aid and has been classified as a drug from plant in the National List of Essential Medicines (NLEM) in Thailand since 2013 (**NLEM, 2013**). Therefore, the active compounds, anti-oxidant, radical scavenging activities of VC plant *in vitro* and catecholamine neurotransmitters, oxidative stress and the mutagenicity effect on chromosome after administrated in rats were the aims of this study.

1.2 Purposes of the study

This study was designed to study the antioxidant activities and active compounds of *Veronia cinerea* Less. extracts *in vitro* and evaluated the activity on catecholamine neurotransmitters, oxidative stress and the mutagenicity effect on chromosome in rats. The specific objectives of this study are as follows:

Specific aim 1: To evaluate the antioxidant activities and active compounds in VC extracts *in vitro* model.

1.1: To compare the antioxidant activities (total antioxidant capacity; TAC), scavenging activity on the nitric oxide (NO), superoxide radical ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$) in among of the VC stem, flower and leaf extracts.

1.2: To evaluate the active compounds as total phenolic, total tannin, catechin, isoflavone, flavonoid, nitrite, nitrate, nicotine and caffeine in among of the VC stem, flower and leaf extracts.

Specific aim 2: To investigate the activity of VC extracts on catecholamine neurotransmitters, oxidative stress and the mutagenicity effect on chromosome in rat model.

2.1: To evaluate the activity of VC extracts from the stem, flower and leaf on catecholamine neurotransmitters (dopamine, noradrenaline and adrenaline) in nicotine-treated rats.

2.2: To evaluate the activity of VC extracts from the stem, flower and leaf on the plasma total antioxidant capacity (TAC) and lipid peroxidation (MDA) in nicotine-treated rats.

2.3: To evaluate the mutagenicity effect of VC extracts from the stem, flower and leaf on the chromosome aberration in rats.

1.3 Scope of the study

In vitro study, each VC extracts were prepared by water-boiled system to condensed juices, before freeze drying. Firstly, extracts from stem, flower and leaf were tested their antioxidant activities with total antioxidant capacity (TAC) and scavenging activity on NO, $O_2^{\bullet-}$ and $OH^{\bullet}$ radicals was also evaluated by spectrophotometry. Secondly, extracts from stem, flower and leaf were analyzed their active compounds as total phenolics and total tannin by spectrophotometry and catechins (catechin; C, epicatechin; EC, epigallocatechin gallate; EGCG, epigallocatechin; EGC and epicatechin gallate; ECG), flavonoid (kaempferol, quercetin, myricetin), isoflavone (daidzin, genistin), nitrite, nitrate, nicotine and caffeine by high-performance liquid chromatography (HPLC).

In rat model; male and female Wistar rats (5-8 weeks of age, 250-300 g) were divided randomly into six groups; control, nicotine, nicotine with bupropion, nicotine with different extracts from leaf, flower or stem groups. After orally fed with extracts with co-injected the nicotine subcutaneously for 20 days, their blood were collected to evaluate the levels of dopamine, nor-adrenaline and adrenaline, including lipid peroxidation (malondialdehyde; MDA), total antioxidant capacity (TAC). Lastly, the mutagenicity effect in chromosome from mitotic index and chromosome aberration after single dose administrated with each extracts was analyzed.

1.4 Hypothesis of the study

1.4.1 Total antioxidant capacity (TAC) and scavenging activities on the nitric oxide, superoxide radical and hydroxyl radical in among of the VC stem, flower and leaf extracts were differently presented.

1.4.2 The active compounds as total phenolic, total tannin, catechins, isoflavone, flavonoid, nitrite and nitrate, nicotine and caffeine in among of the VC stem, flower and leaf extracts were differently presented.

1.4.3 The catecholamine neurotransmitters as dopamine, noradrenaline and adrenaline in among of different rat groups were different presented.

1.4.4 The total antioxidant capacity (TAC) and lipid peroxidation (MDA) levels in plasma in among of different rat groups were differently presented.

1.4.5 The mutagenicity effect on chromosome either mitotic index or chromosome aberration from orally a single dose administration with different VC extracts was not presented.

1.5 Anticipated outcomes

From over all in this study, the outcomes were:

1.5.1 Total antioxidant capacity (TAC) and Inhibition concentration of each VC extracts at 50% on scavenging radicals (nitric oxide, superoxide radical and hydroxyl radicals)

1.5.2 Total phenolic, total tannin, catechins (catechin; C, epicatechin; EC, epigallocatechin gallate; EGCG, epigallocatechin; EGC and epicatechin gallate; ECG), flavonoid (kaempferol, quercetin and myricetin), isoflavone (daidzin and genistin), nitrite, nitrate, nicotine and caffeine.

1.5.3 Catecholamine neurotransmitters (dopamine, noradrenaline and adrenaline levels)

1.5.4 The total antioxidant capacity (TAC) and lipid per oxidation (MDA) levels in plasma.

1.5.5 The percentage of mitotic index and chromosome aberration.