CHAPTER I
INTRODUCTION

1. Rationale and Background

Parkinson's disease (PD) is a second most neurodegenerative disease which has been affected worldwide in all ethnic groups and socioeconomic classes. This disease is commonly found in the elderly person who has age more than 65 years old (Moghal *et al.*, 1994; Olanow *et al.*, 1996). Previous studied demonstrated that the incidence rate of PD in the United States was approximately 13 cases per 100,000 person per year (Tanner and Goldman, 1996). There was no the exact report about the incidence and prevalence of PD patients in Thailand. In 2008, the Medical Statistics of Prasat Neurology Institute of Thailand reported that the number of outpatients of PD was 130 cases (0.07%) (Medical Statistics of Prasat Neurology Institute, 2008). In addition, Chulalongkorn Comprehensive Movement Disorder Centre reported that the number of PD patients in Thailand was approximate 1% of aging population (Chulalongkorn Comprehensive Movement Disorder Centre, 2007). It was also reported that Thai aging population in 2007 was 7,038,000 (National Statistical Office, 2007). Therefore, the estimated prevalence of PD in Thailand will be approximate 70,380 cases.

Parkinson's disease is characterized by bradykinesia, tremor, rigidity and impaired postural reflexes. In addition to the motor symptoms, mental disorders like depression or psychosis, and autonomic and gastrointestinal dysfunction may occur, all of these disorders considerably impair the quality of life of PD patients and also make the burden of caregiver and family (Schrag *et al.*, 2000). The economic impact of PD is varied in different countries. In 2003, Findley and colleagues reported that the direct cost per individual with PD in the United Kingdom was $4,380 (Findley *et al.*, 2003). PD produces not only the high economic burden but also social burden for both the patient family and careers. Based on the increasing prevalence of this disease with the advancing age, the Parkinson's related problem is increasing its important nowadays.

There are many risk factors contributing PD, including age, gender, genetic predisposition, trauma, toxicant exposure. According to Thailand is an agricultural country thus the farmers have high risk to contact chemicals of the pesticide. Previous studies demonstrated that farming particularly pesticide exposure has the strong relationship with PD pathogenesis (Ascherio *et al.*, 2006; Firestone *et al.*, 2005; Kamel *et al.*, 2007; Zorzon *et al.*, 2002).

It is well established that PD is associated with the progressive loss of dopaminergic neuron in the substantia nigra. However, the precise etiology which is responsible for this is still not well understood. Recently, it has been demonstrated that oxidative stress plays an important role in PD pathogenesis which may occur either by the increased excess free radical exposure or by the decreased free radical buffering system (Jenner and Olanow, 1996). The degeneration of dopaminergic neuron leads to the decrease of available dopamine in the brain. Therefore, the main purpose of treatment is focused on the increasing dopamine. Nowadays, the standard drugs for PD treatment are levodopa, dopamine agonist and monoamine oxidase type B inhibitor but unfortunately, these drugs are expensive and produce abundant severe side effects (Cotzias *et al.*, 1969; Factor, 2004; Waters, 1992).

Due to the advance of biotechnology, stem cell implantation has gained much interest for PD treatment (Correia *et al.*, 2005). However, the cost expenditure for this strategy is very high and the procedure is invasive. Moreover, the stem cell that will be implanted to the patients are not specific enough to differentiate to dopaminergic neuron and exert the function compensate to the old neurons. In addition, the deep brain stimulation is also reported to be effective in treating PD (Benabid *et al.*, 2006). However, this strategy is also expensive and usually recommended for the late state of patients who are not response for drug treatment.

Therefore, the development of novel protective and therapeutic strategies against this disease which have low cost, non-invasive, and produce less side effects are still required. Recent data showed that free radicals play an important role on the neurodegeneration in PD and antioxidants could protect against this condition (Prasad *et al.*, 1999). Therefore, the neuroprotective effect of substance possessing antioxidant is focused.

Thailand abounds with many medicinal plants, fruits, and vegetables which can provide many beneficial effects on health. Among various interesting plants, onion (Allium cepa L.) is the most important vegetables worldwide with the high amount consumption because it contains richest flavonoid especially quercetin. Moreover, this vegetable has been grown in many provinces of Thailand. The Office of Agricultural Economics reported that Thailand produced onions 44,977 tons in 2008 (Office of Agricultural of Economics, 2009). Previous study reported that the total flavonols was found about 415-1917 mg per kilogram of fresh weight of red onions (Slimestad *et al.*, 2007) and the total quercetin content in the edible portion of onions varies, but has been noted as high as 345 mg/kg (Bilyk *et al*., 1984; Patil *et al*., 1995; Patil and Pike, 1995).

Quercetin is reported to produce biological actions including antivirus (Ohnishi and Bannai, 1993), antiulcer (Alarcon de la Lastra *et al.*, 1994), anti-inflammatory, antineoplastic and cardioprotective activities (Middleton *et al.*, 2000), and anticancer agent (Lamson and Brignall, 2000). Moreover, it is known to have a strong free radical scavenger (Deschner *et al.*, 1991; Takahama, 1988). Due to its polyphenolic hydroxyl groups, quercetin exhibits its antioxidant property and it contributes significantly to the protective effects of neuronal cells from oxidative stress-induced neurotoxicity, such as Alzheimer's disease (Heo and Lee, 2004). Based on its antioxidant and anti-inflammatory effect, quercetin may be served as a potential therapeutic agent against PD. However, the scientific evidence to support the effect of quercetin on Parkinson's disease is still very limited until now.

To date, it has been demonstrated that the therapeutic drugs used in PD are very limited due not only to the poor absorption in the gastrointestinal tract, but also to short acting and the influence of first pass effect (Jenner, 2008; Swart and De Zeeuw, 1992). Therefore, transdermal preparation has been focused in order to overcome the burden from such problems. Nowadays, the nanofiber is one of interesting strategies to develop new drug administration. Previous study has developed the quercetin loaded-chitosan nanoparticles and determined its antioxidant effect in vitro, they found that this form of quercetin may be useful in improving the bioavailability of quercetin (Zhang *et al*, 2008). At present, the reports about the development of the antioxidant loaded nanofiber (Taepaiboon *et al.*, 2007; Sikareepaisan *et al*., 2008) and

its effective are still very limited. However, less scientific evidence about the production and the determination of efficacy of the antioxidant loaded biodegradable nanofiber are less available.

In Thailand, plenty of onions have been planted in many provinces. Quercetin, a flavonoid found in onions might have the potential to be developed as neuroprotective agent in order to increase the value of onions. Based on the role of free radicals to induce neurodegeneration of dopaminergic neuron, the antioxidant activity and the potential benefit of transdermal route, the development and evaluation of novel transdermal patch of quercetin-loaded nanofiber are focused. Unfortunately, there is no supported scientific evidence until now. Therefore, the current study is designed to determine the efficacy of quercetin biodegradable nanofiber patch on neuroprotective effect against Parkinson's disease.

2. Objectives of the Study

2.1 To determine the protective effect against Parkinson's disease of conventional quercetin administration.

2.2 To determine the protective effect against Parkinson's disease of transdermal biodegradable zein-based polymer loaded with quercetin.

2.3 To compare the effectiveness of conventional quercetin and transdermal biodegradable zein-based polymer loaded with quercetin.

2.3.1 Compare the density of neuron and tyrosine hydroxylase positive neuron in substantia nigra.

2.3.2 Compare the effect of quercetin administered by different intervention on free radicals and antioxidant enzymes activities.

3. Scope and Limitation of the Study

3.1 All animals used in this experiment were Wistar male rats and the age was approximately at 8 weeks old.

3.2 All injections should be done at the same period to avoid the influence of circadian rhythm.

3.3 All stress should be avoided to prevent the influence of stress.

3.4 All experiments, control and experimental groups should be performed in parallel at the same period to avoid effects of seasonal changes.

4. Hypothesis

4.1 If quercetin in a conventional delivery form can protect against Parkinson's disease, the animals which received quercetin should show better motor behavior, cognitive performance and less neuronal damage than those of vehicle treated group.

4.2 If transdermal biodegradable zein-based polymer loaded with quercetin can protect against Parkinson's disease, the animals which received quercetin loaded zein-based polymer should show better motor behavior, cognitive performance and less neuronal damage than those of vehicle treated group.

4.3 If transdermal biodegradable zein-based polymer loaded with quercetin can protect against Parkinson's disease better than conventional quercetin, rats which received transdermal biodegradable zein-based polymer loaded with quercetin should show lower malondialdehyde but higher activities of superoxide dismutase, catalase and glutathione peroxidase than those which received conventional quercetin.

5. Anticipated Outcome

5.1 To provide the information about the protective effect against Parkinson's disease of quercetin.

5.2 To provide the information about efficacy of quercetin administered by various forms and routes.

5.3 To provide the information about the possible underlying mechanism of the difference in efficacy of quercetin administered by various forms and routes.